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TUBERCULOSIS IN GUINEA PIGS*

A SKIN TEST FOR PREEXISTING TUBERCULOSIS IN GUINEA PIGS USED FOR LABORATORY DIAGNOSIS OF TUBERCULOSIS

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THE guinea pig is universally accepted as the animal of choice for the inoculation diagnosis of tuberculosis. Much reliance is placed on the outcome of this procedure. It is always accepted as conclusive in cases of doubt where some evidence of tuberculosis is found in the guinea pig on necropsy with little realization as to whether or not there was a preexisting tuberculous infection.

Two years ago we concluded experiments, the results of which at least to us were surprising. We then found, much to our chagrin, that we had been preceded in this work by at least a decade. In view of the fact that there is little cognizance of their results and conclusions and that experiments with guinea pigs are still being carried on without ascertaining whether the guinea pig is tuberculosis free, we feel that publication is quite apropos.

We also have reference to some of the work on the filterable virus in tuberculosis.[4] The filtrate was injected into the guinea pig without eliminating a preexisting tuberculous focus. If there was such a focus present (and our work has led us to believe that in a large percentage of cases there is), the filtrate would activate that focus thereby causing an erroneous conclusion namely that that filtrate had produced the disease. This has its analogy in the tuberculin tests in cattle. Furthermore at that time (two years ago) we made a survey of the pathologic departments of all the larger hospitals in New York City to ascertain whether an attempt was made to eliminate a

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preexisting tuberculous focus in guinea pigs before doing the inoculation diagnosis for tuberculosis. In not one instance where we had received a reply was the procedure to eliminate an existing focus followed. It is obvious therefore that not only experimentors may come to an erroneous conclusion, but that many human beings may be called tuberculous and serious operations performed such as nephrectomies when only the guinea pig had the tuberculosis. Sewal[1] found spontaneous tuberculosis in a notable proportion of guinea pigs which had been confined in the laboratory, but which had never been intentionally inoculated with tuberculosis. Cooper and Petroff[2] have demonstrated the presence of tubercle bacilli in lymph nodes in 33 per cent of normal guinea pigs. We therefore feel justified in having the following observations published.

Twelve young guinea pigs, supposedly healthy, weighing about 250 gm. were selected for the first experiment and were placed on the floor of the animal room in an enclosure separating them from the other laboratory animals by means of a mesh wire fence. They were allowed to remain there for a period of four days for observation.

On February 26, 1927, an area on the abdomen approximately 2.5 cm. in diameter was bared by plucking the hair so that any skin reaction would be apparent, and the following day the 12 guinea pigs were tested with 0.25 c.c. of a 1-10 dilution of Koch's old tuberculin by intradermal inoculations. After twenty-four hours four of the 12 guinea pigs showed a very slight reaction at the site of the inoculation but it was so indefinite that the results were regarded as doubtful.

Four days later all 12 guinea pigs were retested with 2 minims of a dilution of equal parts of saline and Koch's old tuberculin by intradermal inoculation, and after twenty-four hours no reactions were observed that could be considered definite. Three days after this inoculation, 9 of the 12 retested guinea pigs showed very marked skin reaction; the remaining 3 showed absolutely no reaction. Five of these guinea pigs, 3 (A, B, C) giving negative and 2 (D and E) positive tests, were selected for special observation.

Guinea Pig A (in cage with C and D) showed no skin reaction after test and was used as a control. Died March 15, 1927. Necropsy revealed slight localized inflammation at site of inoculation, enlarged inguinal glands on left side and organs normal in appearance. Microscopic examination of smears made from organs and glands, stained by Ziehl-Neelson's method, failed to demonstrate any acid-fast bacilli.

Guinea Pig B (in separate cage) showed no skin reaction after first test and on March 7 was inoculated subcutaneously with washed live organisms from a pure culture of Mycobacterium tuberculosis (hominis) and on retesting gave positive skin reactions. Died April 22, 1927. Necropsy revealed no abnormal conditions of organs but enlarged inguinal glands on both sides. Microscopic examination of smears made from organs and glands, stained by Ziehl-Neelson's method, demonstrated numerous acid-fast bacilli in all slides.

Guinea Pig C (in cage with A and D) showed no skin reaction after test and on March 7 was inoculated subcutaneously with washed killed organisms from a pure culture of Mycobacterium tuberculosis (hominis), (organisms

placed in autoclave and subjected to the heat of flowing steam for one hour), on retesting gave negative skin reaction. Died March 22, 1927. Guinea pigs B and C were retested on the same day (eleven days later). Necropsy revealed organs and glands in apparently normal condition. Microscopic examination of smears made from organs and glands, stained by Ziehl-Neelson method, failed to demonstrate any acid-fast bacilli, but adjacent glands showed a fairly typical focus, simulating tuberculosis.

Guinea Pig D (in cage with A and C) showed marked skin reaction after test and was used as a control. Died March 20, 1927. Necropsy revealed organs in apparently normal condition but enlarged inguinal glands on both sides. Microscopic examination of smears made from organs stained by Ziehl-Neilsen method, failed to demonstrate any acid-fast bacilli in the organs, but showed typical acid-fast bacilli in the lymph nodes.

Guinea Pig E (in pen in animal room) showed marked skin reaction after test and was used as a control. Died March 19, 1927. Necropsy revealed slightly enlarged, but practically normal inguinal glands and organs, with the exception of right lung, in apparently normal condition. Right lung at base showed white cheesy area. Microscopic examination of smears made from organs and glands stained by Ziehl-Neelson method, failed to demonstrate any acid-fast bacilli in organs or glands with the exception of smear made from right lung. This slide showed typical acid-fast bacilli.

Twelve additional young guinea pigs, supposedly healthy, weighing about 250 gm. and bought from a different concern were placed on the floor of the animal room under the same conditions as the previous lot. They were allowed to remain there for a period of four days for observation.

March 30, the 12 guinea pigs were prepared in a similar manner to the first lot and tested with 2 minims of a solution of equal parts of Koch's old tuberculin and saline by intradermal inoculation. After twenty-four hours 4 of these guinea pigs showed a slight redness at site of inoculation which might be interpreted as the beginning of a reaction but all 4 were doubtful. The other 8 were frankly negative. Forty-eight hours after inoculation 3 of these doubtful guinea pigs developed a positive skin reaction and the other 9 were negative. Some of these animals were killed by the dog in the animal room; the rest were used for subsequent experiments.

DISCUSSION

It will be observed that 75 per cent of the first lot and 25 per cent of the second lot gave positive skin reactions. While the results of the second lot confirmed those of the first, we were unable to follow the same procedure because they were used for subsequent experiments, results of which will be published at some future time. We must summarize the results from the originally selected lot of guinea pigs, however, limited in number, for we feel that it is of sufficient interest to justify publication. The injection of killed organisms did not produce any skin sensitiveness to tuberculin in guinea pig C, although Lange[3] states a transient hypersensitiveness can sometimes be obtained from the injection of a large amount of killed culture. The results in guinea pigs D and E were most interesting and on the findings

of these animals, in addition to the above experiments, we feel that we may emphasize the necessity of testing guinea pigs with tuberculin to exclude a tuberculous focus before using them for laboratory diagnosis or experiments.

Guinea pigs D and E both showed marked skin reactions after tests and were kept under observation and used as controls. On necropsy these animals revealed typical tuberculous lesions in which the acid-fast bacilli were found. Guinea pig B, giving negative skin reaction, was inoculated with washed live organisms and subsequently gave a positive skin reaction, and showed the same pathology on necropsy as guinea pig D which gave a positive skin reaction on all tests and was used as a control, emphasizing that there was a preexisting focus in A similar to that produced in B by artificial inoculation.

Furthermore, necropsies were performed on some of the guinea pigs that had previously given a positive tuberculin test but died for reasons unknown, and in not one instance did we fail to find evidence of tuberculosis.

CONCLUSIONS

1. Tuberculosis is quite prevalent in apparently normal guinea pigs.

2. An intracutaneous tuberculin test reveals the presence of a tuberculous lesion in guinea pigs.

3. Guinea pigs injected with dead tubercle bacilli did not develop any sensitivity to tuberculin.

4. Guinea pigs may have a preexisting tuberculous focus similar to that induced by inoculation.

5. Guinea pigs of uncertain history should be tested with tuberculin before being employed for the inoculation diagnosis of tuberculosis and other laboratory experiments relative to tuberculosis.

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PLASMA PROTEIN IN RELATION TO SUSPENSION STABILITY OF ERYTHROCYTES AND PRECIPITATION OF SERUM PROTEIN WITH ALUMINUM SULPHATE*

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THE sedimentation rate of erythrocytes and numerous serum precipitation (flocculation and ring) tests have long been used for determining the presence of infection in the human subject. Numerous experiments indicate that the etiology of an increased sedimentation rate of erythrocytes is invested in the plasma rather than the cells, since upon transferring cells of normal blood to plasma obtained from a diseased individual, the phenomenon of an increased sedimentation rate is observed. However the rate of sedimentation is within normal limits if cells from a diseased individual are added to plasma from a normal subject.

The observation that protein can be precipitated from serum of the diseased with smaller amounts of the precipitating agent than is possible with the serum of the normal person, has been variously explained as being due to an alteration in the distribution of the serum proteins as might be observed with relative or absolute increases or decreases in the several fractions of serum protein, to the presence of certain lipids and to variation in content of electrolytes. This investigation was undertaken in an effort to determine whether the velocity of sedimentation of erythrocytes, and the precipitability of serum protein as determined by the addition of aluminum sulphate, could be correlated, with quantitative relationship of the various plasma proteins.

That an increased rate of sedimentation of the erythrocytes obtains in the blood of the tuberculous and that the sera of such individuals exhibits an increased precipitability with aluminum sulphate, has been previously reported by the author.[1, 2, 3]

This investigation involved the estimation of fibrin, globulin, and albumin of plasma; sedimentation rate of erythrocytes in citrated blood; and the quantitative relationship of aluminum sulphate to the precipitation of serum protein of human blood. Since normal values for these blood properties must be known before it is possible to make any inference regarding pathologic conditions, examinations were made upon the blood of 20 normal individuals to establish a basis for comparison. In addition, this report incorporates the values for these blood properties as observed in the blood of 20 individuals suffering from such conditions as hypertension, carcinoma, acute infections, ascites, and tuberculosis.

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METHODS EMPLOYED

Blood specimens were collected from an arm vein (without stasis) after a fasting period of fourteen hours. A portion of the specimen was oxalated, the clear plasma separated, and fractionation of the protein was promptly undertaken. Fibrin was separated by recalcifying diluted plasma, and collected on a small glass rod. Globulin was coagulated with 1.5 molar sodium sulphate and removed by filtration, the nitrogen of the filtrate representing that contained in the albumin and nonprotein constituents. Nonprotein-nitrogen was determined as contained in the tungstic acid filtrate. These fractions were quantitatively determined by calculation from the amount of nitrogen recovered after Kjeldahl digestion.

In determining the erythrocyte sedimentation rate (ESR) three parts of blood were mixed with one part of 3.8 per cent sodium citrate solution. Serologic pipettes of 1 c.c. capacity, delivery type (1 c.c. volume contained in a lineal distance of 135 mm. from 0 to 1.0 graduation marks) were filled with the citrated blood by aspiration and the delivery end sealed with beeswax. The pipettes were then set aside in a vertical position at room temperature (approximately 20° C.) and readings made at one-half, one, and two hour intervals, the upper surface of the corpuscle layer serving as a guide from which to record the descending cell column, using the pipette graduations as a convenient scale for recording the extent of fall at the various time intervals. The cell volume of the blood was determined with the Van Allen hematocrit tube. The volume of the individual corpuscle was determined according to the following relation:

$$\text{Volume of corpuscle c.mm.} = \frac{\text{Cell volume per cent}}{\text{Number cells per c.mm.}}$$

For the flocculation test a portion of the blood specimen was set aside for an hour and a half to insure complete clotting after which the serum was separated by centrifuging, and collected. Various amounts of aluminum sulphate (ranging in concentration from 0.02 to 0.12 per cent) contained in a unit volume of 1 c.c. were added to 0.2 c.c. of the blood serum in small test tubes. The serum and reagent were carefully mixed by inverting the tube three or four times. It was observed that more extensive shaking affected only the character of the precipitate as indicated by the formation of somewhat larger flocculi and even very extensive shaking of the mixture was without result upon the incidence of precipitation. The tubes were set aside at room temperature (approximately 20° C.) for one and one-half hours, prior to the reading. Preliminary experiment revealed that incubation at a higher or lower temperature, as 37° C., or 10° C., affected the degree of precipitation quite irregularly, consequently all tests were made at or near 20° C., as a standard procedure. In reading the tubes, a heavy flocculent precipitate that settled out, leaving a clear supernatant fluid was recorded as "xxxx" while a very small amount of flocculent precipitate was recorded as a "x" reaction. Precipitates intermediate in amount were recorded as "xxx" and "xx" reactions according to the amount and density. Uniformity in readings was soon attained after a moderate amount of experience in examining the tubes. Atypical types of flocculation were only rarely observed.

Sera eventually yielding a heavy precipitate usually gave a prompt turbidity upon mixing the serum and reagent. In some instances a definite turbidity appeared within as short an interval as fifteen minutes. However it did not seem feasible to evaluate the precipitability of the serum by the length of the incubation period required for the appearance of a turbidity or definite precipitate, as has been suggested recently by Bodon,[4] as many of the sera yielding heavy precipitates within an incubation period of one hour and a half did not exhibit a definite turbidity immediately upon mixing serum and reagent.

Protein determinations herein reported are based upon plasma rather than upon serum, which was used in all of the precipitation experiments. Concentration of the globulin and albumin fractions in plasma is not entirely comparable to the concentration obtaining in serum. However the possible discrepancy as determined by numerous experiments, is not of great magnitude.

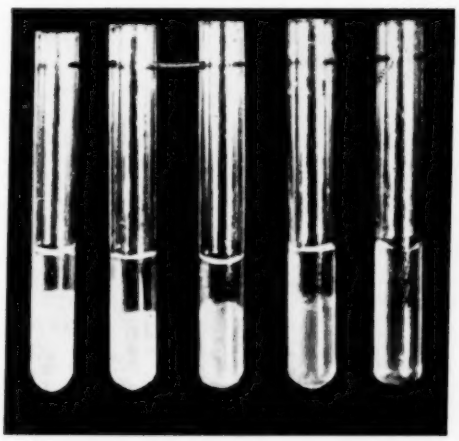

Fig. 1.—Precipitation of serum protein with aluminum sulphate. Reading from left to right, "xxxx", "xxx", "xx", "x", and negative.

The trend of significant alteration in the protein-quotient of serum is readily established by determination of the globulin and albumin contained in the plasma.

EXPERIMENTAL

Since the interpretation of any alteration in these blood properties as observed in the diseased individual, must be predicated upon a conception of values obtaining in the normal state, the results obtained in the examination of the venous blood of 20 normal men are given in Table I.

The values for total protein and protein fractions as obtained in this study are not at great variance with the commonly accepted standards for individuals of this class, although in the results observed wide variation may exist between individuals. Sedimentation readings of 0.13 and 0.27 were the maximum values observed for the one- and two-hour periods respectively. Hence it may be assumed that values significantly greater than these in this class of individuals would be indicative of an accelerated ESR. Variability in the sedimentation rate is not readily correlated with variation in the quan-

TABLE I

NORMAL INDIVIDUALS

SUBJECT	LRJ	OEH	AJR	PJS	ELS	BGM	LFH	BBG	PCH	HS	FLF	FJR	TJR	CAS	EED	WOC	OHR	VCP	DJT	FAG
AGE—YEARS	31	22	23	22	21	24	25	22	23	22	24	23	24	20	22	24	24	20	22	23
WEIGHT—POUNDS	165	210	170	150	155	145	152	172	130	155	175	150	142	150	180	130	185	135	170	145
Plasma—100 c.c.																				
Total protein gm.	6.96	7.87	6.45	7.12	6.43	6.30	5.86	6.94	8.42	7.50	6.31	6.48	7.03	7.21	7.15	6.73	7.58	6.81	7.36	6.66
Fibrin gm.	0.27	0.26	0.30	0.23	0.26	0.26	0.18	0.23	0.26	0.31	0.28	0.26	0.25	0.27	0.26	0.25	0.30	0.29	0.28	0.20
Globulin gm.	2.48	1.51	2.00	2.46	1.68	1.41	1.40	2.05	2.96	2.70	1.72	1.94	2.66	2.35	2.09	1.96	2.54	1.05	2.34	1.80
Albumin gm.	4.21	6.10	4.15	4.43	4.49	4.63	4.28	4.65	5.20	4.50	4.31	4.28	4.12	4.59	4.80	4.52	4.74	5.47	4.74	4.66
Protein Quot. $\frac{\text{Albumin}}{\text{Globulin}}$	1.6	4.0	2.0	1.8	2.6	3.2	3.0	2.2	1.7	1.6	2.5	2.2	1.5	1.9	2.2	2.3	1.8	5.2	2.0	2.5
Erythrocyte Sedimentation Rate																				
30 min.	0.01	0.03	0.02	0.02	0.02	0.05	0.01	0.01	0.02	0.01	0.02	0.01	0.03	0.05	0.02	0.04	0.03	0.05	0.05	0.06
1 hr.	0.02	0.06	0.06	0.04	0.04	0.11	0.06	0.03	0.06	0.03	0.06	0.04	0.07	0.10	0.04	0.09	0.08	0.11	0.10	0.13
2 hr.	0.03	0.13	0.16	0.10	0.19	0.24	0.13	0.08	0.14	0.12	0.17	0.10	0.15	0.23	0.16	0.17	0.16	0.21	0.21	0.27
Erythrocytes, millions per c.mm.	4.98	5.26	4.64	5.20	4.70	5.20	4.76	5.12	5.17	5.64	5.38	5.20	4.68	4.68	5.65	5.48	6.00	4.96	5.20	6.00
Cell Volume-Hematocrit per cent	50.50	43.50	45.00	44.00	47.00	44.5	43.5	55.0	47.0	49.0	53.0	50.0	52.5	53.0	56.0	56.5	61.0	50.0	53.0	50.0
Volume of corpuscle c.mm. x 10^{-8}	10.0	8.2	9.7	8.4	10.0	8.5	9.1	10.0	9.1	8.6	9.8	9.6	11.0	11.0	9.9	10.0	10.0	10.0	10.0	8.3
Serum precipitation with aluminum sulphate. Per cent																				
0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.03	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.04	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.05	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.06	–	–	–	–	–	–	–	–	x	–	–	–	–	x	x	–	x	–	x	x
0.07	–	–	x	x	x	–	x	x	xx	–	–	–	x	x	x	–	x	x	xx	xx
0.08	–	x	x	xx	x	x	x	x	xx	–	–	–	x	xx	xx	–	xxx	x	xx	xx
0.09	xx	xx	xx	xx	xx	x	xx	xx	xxx	x	x	x	xx	xx	xx	xx	xxx	x	xxx	xxxx
0.10	xxxx	xxxx	xx	xx	xxx	xx	xx	xx	xxx	xx	xx	xx	xxxx	xxx	xx	xxxx	xxxx	xx	xxx	xxxx
0.11	xxxx	xxxx	xxxx	xxx	xxxx	xxxx	xxx	xxx	xxxx	xxxx	xxx	xxx	xxxx	xxxx	xxx	xxxx	xxxx	xxx	xxxx	xxxx
0.12	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx

TABLE II

HUMAN BLOOD IN MORBID CONDITIONS

CASE	ML	FD	JG	EDM	WC	GM	MG	GB	DD	MW	MH	HGR	SGH	FBI	EW
SEX	M	F	M	M	M	F	M	M	F	F	F	M	M	M	F
AGE—YEARS	71	48	70	59	62	58	50	45	58	57	16	47	45	23	42
WEIGHT—POUNDS	175	90	130	140	150	115	150	130	125	200	120	140	150	125	140
	CARCINOMA	RECTAL ABSCESS	MYOCARDIAL INSUFFICIENCY	P. CIRRHOSIS ASCITES	SYMPTOMATIC PURPURA	LUNG ABSCESS	CARCINOMA	HYPERTENSION NEPHRITIS	CHRONIC PICKS DISEASE	HYPERTENSION NEPHRITIS	LYMPHATIC LEUCEMIA	PULMONARY TUBERCULOSIS FAR ADVANCED	SULPHEMO-GLOBINEMIA	PULMONARY TUBERCULOSIS MOD. ADVANCED	HYPERTENSION NEPHRITIS
Plasma—100 c.c.															
Total proteins gm.	6.46	8.71	6.48	6.17	6.65	7.90	6.63	6.81	5.01	8.69	8.93	7.67	7.78	7.54	7.33
Fibrin gm.	0.44	0.66	0.27	0.27	0.41	0.79	0.31	0.32	0.29	0.61	0.36	0.54	0.45	0.41	0.29
Glob. gm.	2.95	3.99	2.59	2.91	2.90	3.04	2.54	2.68	1.87	3.18	4.13	2.55	2.38	2.27	1.54
Alb. gm.	3.07	4.06	2.86	2.99	3.34	4.07	3.78	3.81	2.83	4.90	4.44	4.58	4.95	4.86	5.50
Prot. Quot. $\frac{\text{Albumin}}{\text{Globulin}}$	1.0	1.0	1.0	1.0	1.1	1.3	1.4	1.4	1.5	1.5	1.7	1.7	2.0	2.1	3.5
Erythrocyte Sedimentation Rate															
30 min.	0.40	0.56	0.01	0.16	0.50	0.38	0.25	0.02	0.08	0.07	0.06	0.40	0.02	0.15	0.01
1 hr.	0.54	0.66	0.02	0.33	0.73	0.60	0.39	0.12	0.13	0.20	0.20	0.53	0.04	0.28	0.03
2 hr.	0.62	0.71	0.08	0.45	0.83	0.69	0.53	0.18	0.21	0.32	0.50	0.57	0.09	0.38	0.10
Erythrocytes per c.mm. x 10^6	3.72	3.67	5.30	3.47	1.24	4.30	4.00	5.59	4.54	4.83	2.90	4.10	6.08	5.30	4.46
Cell Volume-Hematocrit per cent	38.5	33.0	58.0	46.0	14.0	40.0	40.5	64.0	59.0	58.5	25.0	38.0	48.0	52.0	49.0
Volume of corpuscle c.mm. x 10^{-8}	10.0	8.9	10.9	13.0	11.0	9.3	10.0	11.0	12.0	12.0	8.6	9.2	7.8	9.8	10.9
Serum precipitation with aluminum sulphate. Per cent															
0.02	–	–	–	–	–	–	–	–	–	–	–	x	–	–	–
0.03	x	xx	–	–	–	–	x	–	–	x	x	xx	–	–	–
0.04	xx	xxx	–	x	–	–	xx	x	–	xx	x	xxxx	–	xx	–
0.05	xxxx	xxxx	x	x	–	xx	xxx	xxxx	–	xxx	xx	xxxx	–	xxxx	–
0.06	xxxx	xxxx	xx	xx	–	xxxx	xxxx	xxxx	x	xxxx	xx	xxxx	–	xxxx	–
0.07	xxxx	xxxx	xxx	xx	–	xxxx	xxxx	xxxx	xx	xxxx	xxx	xxxx	–	xxxx	x
0.08	xxxx	xxxx	xxxx	xxx	–	xxxx	xxxx	xxxx	xx	xxxx	xxx	xxxx	–	xxxx	x
0.09	xxxx	xxxx	xxxx	xxxx	–	xxxx	xxxx	xxxx	xx	xxxx	xxxx	xxxx	xx	xxxx	xx
0.10	xxxx	xxxx	xxxx	xxxx	xx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxx

titative distribution of plasma proteins or in the concentration of erythrocytes in the blood.

It is significant that precipitability of the blood serum is not correlated with the ratio of albumin to globulin, as contained in the plasma. Considerable variation in precipitability is to be noted among these normal sera. However in no instance did precipitation occur with 0.05 per cent of aluminum sulphate in the reagent, an amount designated by Matefy[5] as indicating an abnormal serum lability of tuberculous individuals.

Further study of the relationship existing between the blood properties that constitute the subject of this investigation was made upon the blood of a small number of human subjects, individually presenting a fairly well-defined type of pathology. No attempt was made to determine the characteristic blood alteration obtaining in any given disease as the number of cases available for study was inadequate for such a purpose. The various clinical diagnoses and the results of these examinations are listed in Table II.

These findings, in many instances, present a departure from the established limits of normal variation that is of considerable magnitude.

DISCUSSION

The data indicate that sedimentability of the blood is not correlated with significant variation in the size of the individual corpuscle. In many instances an increased ESR is observed in blood having a low content of red blood cells so that an anemia per se may upon occasion be a determining factor in this phenomenon. It is apparent that the concentration of erythrocytes in the blood must be considered in evaluating the significance of an increased ESR, as the concentration may have an inverse relationship to the speed of sedimentation.

In the blood of normal individuals sedimentability of the erythrocytes is a variable property and the variation observed between normal individuals is not to be explained upon the basis of quantitative distribution of the respective plasma proteins. (In repeated examinations of the blood of some of these normal individuals, covering a period of several months, the magnitude of periodic variation approached or was equal to that observed between various individuals.)

An increased ESR was observed in the presence of various morbid conditions and in most instances there was also observed either an increase in fibrinogen or a decrease in the ratio of albumin to globulin. Therefore, it seems possible that these factors may independently or jointly be determinative of the ESR.

Minimal concentration of aluminum sulphate effecting protein precipitation in the serum of the normal human subject was determined. Precipitation with a concentration of *less* than 0.06 per cent may be considered indicative of increased precipitability of serum constituents. Precipitation occurred in all of the normal sera at a concentration of 0.09 per cent or *less*. In the presence of various diseases the marked increase in serum precipitability is not correlated with alteration in the ratio of globulin to albumin as contained in the plasma.

In general, the addition of an electrolyte, such as aluminum sulphate to serum, yields a precipitate (often referred to as a metallic albuminate) which is perhaps not a true combination, but a double salt or loose absorption compound of the protein with the salt. This reaction may be considered quasi-reversible in that dilution with water or removal of the salt by dialysis does not restore the changed protein. However, such precipitates are soluble in an excess of the salt solution. Although hydrolysis occurs in an aqueous solution, rendering the solution acid, perhaps the effectiveness of this salt as a precipitating agent is explained on the basis that electro-negative colloids are precipitated by the cation and in dilute solution the flocculation effected is related to or a function of its valence.

It is noted that blood exhibiting the phenomenon of an increased ESR quite regularly exhibits in addition the property of increased precipitability of the serum. This correlation is observed regularly except in conditions wherein the extreme anemia per se may serve to explain the accelerated ESR.

SUMMARY

1. Increased sedimentability of the erythrocytes in the blood of the diseased is often associated with a shift in plasma protein toward the more labile fibrinogen and globulin fractions.

2. Increased precipitability of serum protein, as determined with aluminum sulphate, is observed in the presence of various morbid conditions and may or may not bear relation to the quantitative distribution of plasma proteins.

3. A correlation between sedimentability of red blood cells and precipitability of serum protein is quite regularly observed.

4. Diagnostic value of the aluminum sulphate serum flocculation test, may be enhanced by titration of the serum with graded amounts of the reagent, as herein accomplished.

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CARBOHYDRATE METABOLISM IN ACROMEGALY*

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THE most exhaustive work on this subject is by Davidoff and Cushing.[1] These authors review 100 cases which they have studied and discuss their conception of the mechanism involved in the disturbance of the carbohydrate metabolism. The subject is of importance not only per se, but also because there are certain fundamental principles involved, which have a general application.

Hansemann[2] found approximately 12 per cent among 97 reported cases of patients with acromegaly to be suffering from diabetes mellitus; Hinsdale[3] found 11 per cent in 130 cases; Borchardt,[4] on the other hand, found 35.5 per cent of 176 cases recorded up to 1908. The variability of these percentages doubtless depends a great deal upon what the compiler of the statistics has been willing to accept as "diabetes mellitus." Davidoff and Cushing in their series of 100 personally observed cases of acromegaly found one out of four to have glycosuria, and one out of eight to have clinically outspoken diabetes mellitus. The question is this: "Given a case of glycosuria, what criteria shall one employ to determine its nature?"

It is not within the scope of this paper to discuss the various theories advanced for the explanation of the disturbance of the carbohydrate metabolism in acromegaly. It seems quite certain that to elucidate this problem the relationship of the pancreas to the hypophysis must be determined.

Davidoff and Cushing state the following: "It is conceivable that the oversecretion of the hypophyseal substance may indirectly produce glycosuria in one of two ways: (1) by neutralizing the secretion of the islet tissue in circulation, thereby causing compensatory hypertrophy with ultimate exhaustion of the cells of Langerhans, leading to the degenerative changes with which we are familiar, or (2) by actually checking the secretory function of the islets which might possibly lead to no histopathologic changes. However this may be, it seems wholly improbable that the diabetes in acromegaly is due to an independent and coincidental lesion, functional or otherwise, of the pancreatic islets."

It has been conclusively shown by Evans[5] and his co-workers that acromegaly is an expression of the hyperfunction of the pituitary gland, and more specifically, an oversecretion of the acidophilic cells of the pars anterior.

J. H. Burn[6] has done work to demonstrate an antagonism between insulin and pituitrin. He showed that pituitrin (posterior lobe extract) definitely neutralizes the power of insulin to lower blood sugar; this was not true of

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anterior lobe extracts. He considers this antagonism to be in the nature of a chemical binding of insulin by pituitrin. Corroborative work was done by Davidoff and Cushing.[1] In three of their patients with acromegalic diabetes who had been shown to respond normally to insulin, 20 units of insulin combined with 1 c.c. of solution of pituitary (Pituitrin, P. D. & Co.) were injected. The expected fall in blood sugar did not occur.

An interesting point is this: Why should posterior lobe extracts be those to counteract insulin, when, as far as we know, hyperpituitarism is an anterior lobe disorder? Davidoff and Cushing state that possibly the posterior and anterior lobes have interdependent rather than the quite separate functions generally ascribed to them.

It was stated above that Davidoff and Cushing found glycosuria in one-fourth of their cases, but considered diabetes mellitus to be present only in one-eighth. They also found in a study of seven cases which had no glycosuria, that all had a blood-sugar curve higher than normal. In other words, all cases of acromegaly have a disturbance of carbohydrate metabolism. If the mechanism described by Davidoff and Cushing is the sole one operating, then all cases of acromegaly should have diabetes mellitus, or none should have it.

In discussing carbohydrate metabolism, two phases must be considered: (1) the storage of carbohydrates (glycogenesis and glycogenolysis), and (2) the oxidation of glucose. Thus the administration of adrenalin favors glycogenolysis, with consequent hyperglycemia and glycosuria, and an increase in the oxidation of glucose. Insulin favors glycogenesis and an increase in the oxidation of glucose. It is evident that considered separately insulin and adrenalin have opposite actions as far as the storage of carbohydrates is concerned, but act similarly on the oxidation mechanism.

It would be well to digress for a moment and consider the following point which is closely related to this subject. It is stated that the administration of adrenalin produces a diminution in glucose tolerance, for if adrenalin is given to a susceptible individual who has received 100 gm. of glucose, he will exhibit a glycosuria. The blood-sugar curve will resemble that of a mild diabetic. The respiratory quotient during this period, however, will rise from a fasting level of, let us say 0.80, to almost 1. The amount of glucose oxidized per unit time is greater than in a normal individual, for there is an increase in the amount of oxygen consumed in the individual receiving adrenalin. A mild diabetic, given 100 gm. of glucose, may exhibit the same blood-sugar curve, and the same amount of glycosuria; his fasting respiratory quotient may also be 0.80, but will rise only moderately, let us say to 0.86. Surely it cannot be said that both have a diminished tolerance for glucose. From the teleological standpoint the important phase of carbohydrate metabolism is oxidation. It was shown above that more glucose is oxidized per unit time by an individual receiving adrenalin than by a normal one. The glycosuria is simply incidental to the hyperglycemia. To state then, that the administration of adrenalin engenders a diminution in glucose tolerance is misleading. A deficiency in glucose tolerance should be considered as existing only when there is an impairment in both the ability to oxidize and store glucose.

With this as an introduction, we may now proceed to a discussion of the influence of pituitrin on carbohydrate metabolism. It was stated above that Davidoff and Cushing[1] and Burn[6] found that pituitrin neutralizes the effect of insulin on blood-sugar concentration. The experiments by Cammidge and Howard[7] on the interrelation of insulin and other gland extracts on metabolism are of great value. They showed that the injection of insulin produces an increase in the respiratory quotient in fasting animals, which can be prevented by the administration of pituitrin. Their work on fed animals, however, is of far greater importance. They determined that white rats, after an abstinence of twenty-four hours from food, gave a respiratory quotient of 0.77. After feeding, the respiratory quotient was found to have increased to 0.91. The increase in the respiratory quotient is to some extent due to the conversion of carbohydrate to fat, but in greater part it is the result of the oxidation of glucose. It is logical to assume that this rise in respiratory quotient is a result of the secretion of insulin by the pancreas, for were these animals to be pancreatectomized, the respiratory quotient would not rise on feeding, and marked hyperglycemia and glycosuria would also appear. They found that the injection of ¼ c.c. of pituitrin caused a glycosuria in all animals, *but the respiratory quotient remained 0.91.* In other words pituitrin interfered with the action of insulin on the blood-sugar level, as is evidenced by its producing hyperglycemia and glycosuria, but it in no way interfered with the oxidation of glucose. This fact is of fundamental importance. It contradicts the statement of Burn[6] and of Davidoff and Cushing[1] that pituitrin completely nullifies the action of insulin.

We believe that we are justified in drawing the following conclusions: If an adequate amount of insulin is secreted by the islets, there is no known substance, as far as we are aware, which will interfere with its power to cause the oxidation of glucose. The failure of the respiratory quotient during fasting to rise markedly after the ingestion of large quantities of glucose is evidence of a deficiency of the Islands of Langerhans. Conversely the presence of a respiratory quotient approaching 1 after the administration of large quantities of glucose, is absolute evidence of normally functioning islets, regardless of the extent of hyperglycemia and glycosuria.*

With these facts at hand we may now consider carbohydrate metabolism in acromegaly. Practically all cases of acromegaly have a blood-sugar tolerance curve above normal (Davidoff and Cushing). All other factors being equal the presence of glycosuria will depend simply on the extent of the hyperglycemia. Now the question is: Which of these cases have diabetes mellitus? This can be conclusively answered by studying the respiratory quotient after giving large quantities of glucose. If the respiratory quotient approaches 1 the functional activity of the islets may be considered normal; if the respiratory quotient is much less than 1, then we must conclude that there is an inadequate secretion of insulin by the Islands of Langerhans; this constitutes diabetes mellitus.

In the nondiabetic cases the hyperglycemia will be caused by the excessive

*This does not include phlorizin diabetes where the respiratory quotient rises only slightly after the administration of glucose. This is a result, not of the inability of the organism to oxidize glucose, but of the rapid elimination of glucose through the kidney.

pituitary secretion only; whereas in the diabetics two factors will be operating to produce an increase in the blood-sugar concentration, the increased pituitary secretion and the diminished insulin secretion.

We will now present a patient with acromegaly who came to our diabetic clinic because of the presence of glycosuria. A glucose tolerance test was performed; 150 gm. of glucose were given (Table I). The blood-sugar content was determined by the Kramer Gittelman[8] micro-method (modification of Folin-Wu) in which blood is obtained by pricking the finger tip. It has been shown by Foster[9] that this blood is equivalent to arterial blood. This is

Blood-Sugar Curve

BLOOD SUGAR	FASTING LEVEL	45 MINUTES P.C.*	TWO HOURS P.C.
MG./100 C.C.	202	380	416

Respiratory Quotient Curve

FASTING LEVEL	1½ HR. P.C.	2½ HR. P.C.
0.78	0.78	0.82

*P.C.—After ingestion of 150 grams of glucose.

not significant as far as this work is concerned. The respiratory quotients were determined by Bailey's method. (Tissot spirometer and Henderson-Haldane gas burette.)

This patient is suffering from diabetes mellitus.

It is very interesting to note that although there may be marked glycosuria in acromegalic diabetes, ketosis is often not present, or if present, only of slight degree. This may be explained as follows: As stated above the glycosuria in acromegalic diabetes is due to the sum of two factors: (1) the excessive pituitary secretion and (2) the deficiency in insulin secretion. Thus, although there may be only slight reduction in the amount of insulin secreted, the glycosuria nevertheless will be marked. Since the oxidation of glucose in this case is only moderately affected, ketosis will not result, or be only slight in amount.

It is evident that the work on which our conception is based is meager in amount. It fits in well, however, with all the phases of the question. We believe that continued study of more cases of acromegaly along the lines laid down by us will lead to a compelte elucidation of the problem.

SUMMARY AND CONCLUSION

Although acromegaly is caused by a diseased state of the anterior lobe of the pituitary gland, it is the secretion of the posterior lobe which has been shown experimentally to affect carbohydrate metabolism. Davidoff and Cushing state: "It possibly may come to be shown that the posterior and anterior lobes have interdependent rather than the quite separate functions generally attributed to them."

The disturbance in carbohydrate metabolism in acromegaly may be incidental to it or to concomitant diabetes mellitus.

Of cardinal importance is the fact that although pituitrin neutralizes the effect of insulin on blood-sugar concentration, it does not affect the action of insulin on the oxidation mechanism of glucose.

This fact may be utilized in determining the nature of the carbohydrate disturbance. If the respiratory quotient, after the administration of a large quantity of glucose fails to approach 1, diabetes mellitus is present; if the respiratory quotient does approach unity, the glycosuria is symptomatic regardless of its degree.

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STUDIES ON FAT METABOLISM*

I. Fat Tolerance in Obesity

A Preliminary Study

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SOME forms of obesity have been generally considered as due to some disturbance of fat metabolism, a distinction being made between exogenous obesity, due to overfeeding and inactivity, and endogenous obesity, due to a variety of abnormal physiologic or pathologic functions. Newburgh[1] has recently presented evidence to support the view that the obese subjects, irrespective of disease or constitutional tendency, are obese because they take in more than is used up; or the disease with which it is associated causes obesity by increasing the appetite or decreasing the activity of a formerly active individual. Much study is necessary to prove the correctness or falsity of these views.

Since the study of sugar tolerance and sugar metabolism in diabetes mellitus has proved to be of such value, and since fat metabolism and obesity are intimately related to sugar tolerance and sugar metabolism, and since obesity is so closely associated with the disease of certain organs, studies on fat tolerance in obesity might prove to be of great value.

Our ideas on how fat gets into the blood and how it is oxidized are fairly clear, but only a little is known concerning the intermediate stages of fat metabolism. Bloor[2] has found on the ingestion of a fat meal that the total fatty acids in plasma and corpuscles increase, that the lecithin in the corpuscles increases without much change in the plasma, and that no definite change occurs in the cholesterin. Cowie and Hoag[3] administered fat in the form of cream to normal children and adults. They found that in five children the maximum lipoid content of the blood was reached in from five to seven hours and in three adults at the sixth hour. When the fat was given with a large amount of sugar, they found that the highest lipoid content was reached at the second hour. The relation of carbohydrates to the fat in the diet of the diabetic patient is thoroughly discussed by Joslin,[4] which shows that the two are related in some way. The results of Rabinowitch[5] show that insulin has a marked effect in reducing the blood lipoids in diabetes, and according to Chauffard[5] and Labbe[6] insulin affects fat metabolism independently of its effect on carbohydrate metabolism.

Such observations have caused us to ask ourselves the following questions: Is the alimentary lipemia of an obese subject different from that of a normal subject? Is it possible to differentiate between certain types of obesity on the basis of the alimentary lipemia curve? What factors, and especially do

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hormonal factors, play a rôle in the regulation of the movements of fat in the obese subject? The work presented in this paper is a preliminary attempt to find an answer for these questions. It is possible that a study of obesity from this viewpoint may prove to be of more value than has been the study of basal metabolism, the respiratory quotient and the specific dynamic action of foods.

METHODS

Bloor's method[7] was used for the determination of the total plasma lipoids, cholesterin and fatty acids. Cubital vein blood was drawn and the citrated plasma used. Three samples of the petroleum ether extract were used each time. The results on these samples never varied more than 3 per cent.

The blood fat content of normal and obese subjects was determined fifteen hours (overnight) after the last meal. Marked cases of obesity were selected for this purpose, the average overweight being 95 pounds, and their physical examination and complaint other than obesity being negative.

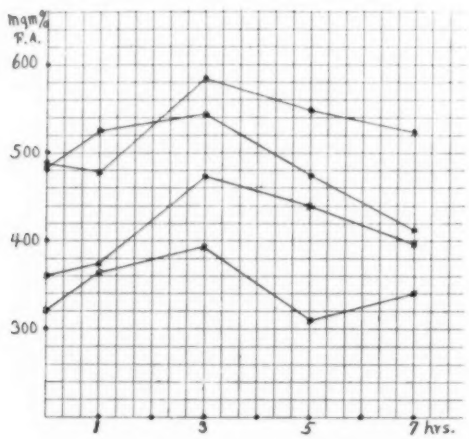

Fig. 1.—Normal fat tolerance curves.

The alimentary fat tolerance test was performed as follows: (1) blood was taken at 9:00 A.M., 15 hours after the last meal; (2) a pint of 20 per cent cream was then given the patient which was followed by approximately 500 c.c. of water; (3) blood samples were drawn at one, three, five, and seven hours after the meal of cream, no food nor water being allowed until after the seven hour blood sample was drawn.

RESULTS

The total fatty acid content of the blood plasma of fifteen normal subjects taken in the morning fifteen hours after the last meal averaged 0.373 per cent. The maximum content was 0.488 per cent, and the minimum was 0.255 per cent. These results compare favorably with those of Bloor whose average was 0.370 per cent and with those of McClure and Huntsinger[8] whose average was 0.364 per cent.

The average cholesterin content of the blood plasma of this normal group was 0.132 per cent, which is definitely lower than Bloor's average. The maximum value in this group was 0.300 per cent, the minimum, 0.072 per cent.

TABLE I

FAT TOLERANCE IN NORMAL PERSONS

NAME OF CASE			GA.	RA.	BL.	O'C.	RY.	MA.	HC.	CA.
Total Lipids Mg. in 100 c.c. Blood Plasma	Before Fat Meal		481	504	625	412	480	414	504	642
	After Fat Meal	1 Hour	489	588	675	449	506	455	591	620
		3 Hours	509	597	697	477	599	491	641	738
		5 Hours	536	521	616	508	578	414	560	682
		7 Hours			552	501	530	439	588	666
Cholesterol Mg. in 100 c.c. Blood Plasma	Before Fat Meal		111	144	140	99	120	92	120	154
	After Fat Meal	1 Hour	111	152	151	119	129	90	129	144
		3 Hours	120	133	151	97	123	96	141	154
		5 Hours	120	140	144	103	134	102	120	133
		7 Hours			140	101	112	96	132	138
Total Fatty Acids Mg. in 100 c.c. Blood Plasma	Before Fat Meal		370	360	485	313	360	322	384	488
	After Fat Meal	1 Hour	378	436	524	330	377	365	462	476
		3 Hours	389	464	546	380	476	395	500	584
		5 Hours	416	381	472	405	440	312	440	549
		7 Hours			412	400	418	343	456	528
Maximum Increase of Fatty Acids		In Per Cent of Initial Value	12	28	12	28	32	23	30	20
		In mg. per 100 c.c. Plasma	46	104	61	92	116	73	116	96

The total fatty acid content of the blood plasma of twenty obese subjects taken in the morning, fifteen hours after the last meal, averaged 0.435 per cent. The maximum value was 0.691 per cent and the minimum 0.225 per cent. The average cholesterin content of the blood plasma of this obese group was 0.128 per cent.

The alimentary fat tolerance tests were performed on 8 normal and eighteen obese subjects.

In the normal subjects (Table I and Fig. 1) the fatty acid content usually shows an increase within one hour after the meal of cream, the peak of the increase occurring from three to five hours after the meal. Seven hours after the meal the fatty acid content was usually less than the five hour content. In these normal subjects the maximum increase after the fat meal was 116 mg. and the minimum 46 mg. per 100 c.c. of blood. Expressed in percentage of the initial fatty acid value the maximum increase was 32 per cent and the minimum 12 per cent.

The cholesterin content of the blood plasma showed no definite or uniform change in these normal subjects nor in the obese subjects, and for this reason the cholesterin values only appear in Table I. Obviously the change in total lipin was due chiefly to the change in fatty acid.

In Fig. 1 the results on subjects Ca., Bl., Ma., and Ry. are shown in the form of a curve. They resemble in a general way a sugar tolerance curve, except for the time factor.

An analysis of the results of the fat tolerance tests in the 18 obese subjects reveals the interesting fact that they can be divided into three groups: Group I (7 cases) in which the fat tolerance curve was like that of normal subjects; Group II (5 cases) in which the fat tolerance might be called "high"; and Group III (6 cases) in which the fat tolerance might be called "low."

Table II shows the results on seven cases in which the results of the fat tolerance test are within normal variations, the increase in fatty acids varying from 48 to 106 mg. or expressed in percentage, an increase varying from 16 to 35 per cent.

TABLE II

FAT TOLERANCE IN OBESITY. (GROUP I.)

Name of Case	TOTAL FATTY ACIDS MG. IN 100 C.C. BLOOD PLASMA					MAXIMUM INCREASE OF FATTY ACIDS	
	Before Fat Meal	After Fat Meal				In Per Cent of Initial Value	In mg. Per 100 c.c. Plasma
		1 Hour	3 Hours	5 Hours	7 Hours		
Al.	296	353	344	328	337	18	48
Gi.	457	459	539	535	486	16	82
Li.	391	398	470	352	333	20	79
Su.	402	384	452	505	413	25	103
My.	465	540	571	501	477	23	106
Za.	333	259	416	361		24	83
Ka.	278	315	329	377		35	99

Table III shows the results on 5 cases in which the results of the fat tolerance test indicate that the tolerance of the patient for fat is high. Three of these 5 obese subjects after ingesting the fat meal showed a decrease in blood

plasma fatty acids varying from 66 to 132 mg.; one, a slight increase the first hour, which was followed by a decrease; the other case showed a maximum increase of 23 mg. or 6 per cent. Fig. 2 shows the curves on 3 of the cases.

Table IV shows the results on the six cases in which the fat tolerance of the subject might be termed "low." The increase in the blood plasma fatty acids

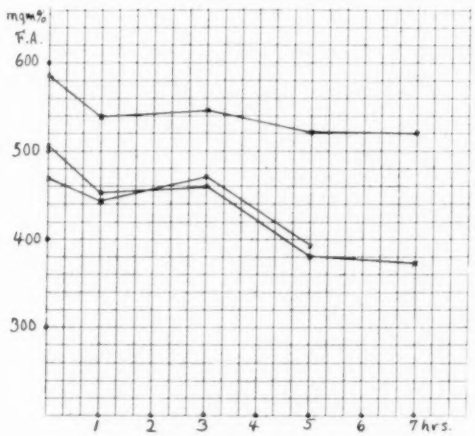

Fig. 2.—High fat tolerance curves.

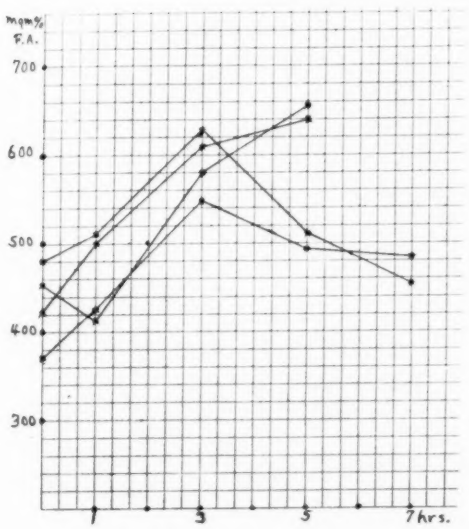

Fig. 3.—Low fat tolerance curves.

varied from 141 to 222 mg. or from 31 to 60 per cent, which is greater than that of the 8 normal subjects and 7 of the obese subjects. Fig. 3 shows the curves on 4 of the cases.

These fat tolerance results suggested that the sugar tolerance of these patients be determined. So in 17 of the 18 cases sugar tolerance tests were made; 1.75 gm. of glucose per kg. body weight was given by mouth, the blood sugar and urine being examined before, and at one, two, and three hours after.

TABLE III

HIGH FAT TOLERANCE IN OBESITY. (GROUP II.)

Name of Case	TOTAL FATTY ACIDS MG. IN 100 C.C. BLOOD PLASMA					MAXIMUM CHANGE OF FATTY ACIDS	
	Before Fat Meal	After Fat Meal				In Per Cent of Initial Value	In mg. Per 100 c.c Plasma
		1 Hour	3 Hours	5 Hours	7 Hours		
Sc.	585	541	555	527	519	−11	− 66
Co.	470	447	470	393		−16	− 77
Wa.	509	449	466	388	377	−26	−132
Gr.	691	719	567	653	631	+ 4	+ 28
						−18	−124
Ha.	386	409	401	397	342	+ 6	+ 23

In the case in which the sugar tolerance was low, the test was repeated with 100 gm. of glucose, regardless of the body weight. The sugar tolerance was considered "low" when the blood sugar mounted to 250 mg. or more and was associated with glycosuria. The sugar tolerance was considered "high" when the blood sugar did not rise above 150 mg. and the initial blood sugar level was resumed at the end of three hours. The sugar tolerance between these extremes was called average. The correlation of the fat and sugar tolerance results is shown in Table V.

TABLE IV

LOW FAT TOLERANCE IN OBESITY. (GROUP III.)

Name of Case	TOTAL FATTY ACIDS MG. IN 100 C.C. BLOOD PLASMA					MAXIMUM INCREASE OF FATTY ACIDS	
	Before Fat Meal	After Fat Meal				In Per Cent of Initial Value	In mg. Per 100 c.c Plasma
		1 Hour	3 Hours	5 Hours	7 Hours		
Go.	452	408	578	655		60	203
Fa.	422	500	611	640		51	222
McG.	476	519	622	583		31	156
Kae.	399	426	525	540	525	35	141
Di.	372	426	548	492	483	47	176
Be.	480	509	634	514	451	32	154

TABLE V

RELATION OF FAT TOLERANCE TO SUGAR TOLERANCE IN OBESE SUBJECTS

	FAT TOLERANCE			SUGAR TOLERANCE		
High	Wa.	Co.	Sc.	Wa.		
	Ha.	Gr.		Wa.		
Average	Li.	Al.		Li.	Co.	Be.
	Su.	Kam.		Su.	Gr.	
	My.			My.		
	Za.			Za.		
Low	Go.		Be.	Go.	Al.	Sc.
	Fa.			Fa.	Kam.	
	McG.			McG.		
	Kae.			Kae.		
	Di.			Di.		

The results shown in Table V demonstrate quite an interesting and striking correlation between the fat and sugar tolerance of obese subjects. Out of the 17 cases there is only one case in which the correlation is contradictory, namely, subject Sc. who shows a high fat tolerance and a low sugar tolerance.

A case from the "high" tolerance group and the "low" tolerance groups will be briefly reviewed.

1. *Case of High Sugar Tolerance and High Fat Tolerance.*—Mrs. Wa., twenty-seven years old, married, has two children. Present weight 298 pounds, height 66 inches, 137 pounds overweight. Patient is a member of a stout family; she was always much overweight. Menses regular, with normal flow. Pulse 90. Blood pressure 120/80. B.M.R.—2 per cent. Sugar tolerance test, after ingestion of 1.75 gm. glucose per kg. body weight, gives the following blood-sugar values: 80 mg. per cent, fasting; 134 mg. per cent after one hour; 151 mg. per cent after two hours; 72 mg. per cent after three hours. Fat tolerance test shows total fatty acids in blood plasma: 509 mg. per cent fasting; 449 mg. per cent after one hour; 466 mg. per cent after three hours; 388 mg. per cent after five hours; 377 mg. per cent after seven hours.

2. *Case of Low Sugar Tolerance and Low Fat Tolerance.*—Mrs. McG., thirty-one years old, married, has one child. Present weight, 298 pounds, height 68 inches, 135 pounds overweight. Patient was always overweight, but gained particularly in the last seven years after an operation when both ovaries were removed. Pulse 72. Blood pressure 178/104. B.M.R.—5 per cent. Sugar tolerance test with 1.75 gm. glucose per kg. body weight: 104 mg. per cent (fasting value); 256 mg. per cent after one hour; 278 mg. per cent after two hours, with 2 per cent sugar in the urine; 256 mg. per cent after three hours, with 1.5 per cent sugar in the urine. Test repeated two weeks later with 100 gm. glucose: 92 mg. per cent (fasting value); 206 mg. per cent with 0.4 per cent sugar in the urine after one hour; 168 mg. per cent after two hours; 132 mg. per cent after three hours. Fat tolerance test: 476 mg. per cent (fasting value); 519 mg. per cent after one hour; 622 mg. per cent after three hours; 583 mg. per cent after five hours.

DISCUSSION

A comparison of the fasting fatty acids of the blood plasma of normal and obese subjects shows that the average fatty acid content of the latter groups is somewhat higher (62 mg.) than the average of the normal group. The maximum and minimum values in the subjects of both groups, however, fall almost within the same limits, which detracts from the significance of the fasting blood fat values in any single obese subject.

The observation that all obese subjects do not react similarly to the fat meal, but may be divided into three groups, one in which the fat tolerance may be called "high," one in which it may be called "average," and the third in which it may be called "low," is highly suggestive and may have a fundamental bearing either on the problem of obesity or on the problem of the relation of obesity to the endocrine glands. Considerable speculation and theorization based on observations in the literature and on known facts might be entered upon at this point in our discussion, but because of the preliminary nature of this paper, it will be omitted. One point might be emphasized, however, namely, the close relation which apparently exists between alimentary lipemia and sugar tolerance. It can be safely stated that in the presence of a normal liver the insulin output of the pancreas is the most important factor controlling

sugar tolerance. The correlation between the fat and sugar tolerance in our subjects indicates that the blood fat is controlled by insulin, in a manner similar to that by which insulin controls blood sugar. This view may be expressed as follows: Just as insulin promotes the formation of glycogen from the circulating blood sugar, insulin also promotes the deposition of tissue fat from the circulating blood fat.

Obviously fat tolerance tests on more obese subjects should be made, and more than one test should be made on each case. Also, tests should be made on "constitutionally lean" subjects. This problem also lends itself to animal experimentation. We are now engaged in performing such experiments.

SUMMARY

1. The average fasting fatty acid content of the blood plasma of obese subjects is somewhat higher than that of normal subjects. This finding does not have much significance in the individual case because of the variations in different cases.

2. The effect of the administration of a pint of 20 per cent cream on the blood plasma lipids of 8 normal and 18 obese subjects was studied. No definite change resulted in plasma cholesterin. The fatty acid content was increased in the normal subjects and a fairly uniform curve resulted, a maximum content being reached from three to five hours after the meal of cream. The results on the 18 obese subjects showed that 7 gave a normal response, and that 11 gave an abnormal response. Of these 11 cases, 5 showed a "high" tolerance, the fatty acid content decreasing after the meal, and 6 showed a "low" tolerance, the fatty acid content increasing more than 116 mg. or the highest of the normal cases.

3. A correlation exists between the "sugar tolerance" and the "fat tolerance" in obese subjects. If the sugar tolerance is high, the fat tolerance is high; if the sugar tolerance is low, the fat tolerance is low; if the sugar tolerance is "average," the fat tolerance is "average."

We desire to express our thanks to Dr. A. C. Ivy for his interest and advice.

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THE RELATIONSHIP OF POTASSIUM TO THE DEPRESSOR EFFECT OF LIVER EXTRACT*

By Ralph H. Major, M.D., and C. J. Weber, Ph.D., Kansas City, Kans.

GOERNER and Haley in a recent article on the nature of the depressor substance in hepatic extract, state that, "as a result of our investigations on liver extract, we believe that the depressor substance is monopotassium dihydrogen phosphate with possibly traces of other potassium salts aiding this action. It would also seem that the potassium ion is the active depressor agent, since other phosphates fail to cause depression and other potassium salts react positively."

Goerner and Haley obtained from liver extract a batch of crystals which, when purified, proved to be crystals of KH_2PO_4 and which, when injected into cats, produced a marked fall in blood pressure, often with death of the animal. In a typical experiment they employed a solution containing 0.224 gm. of KH_2PO_4 dissolved in 1 c.c. water (a 22 per cent solution) and obtained with a dose of 0.25 c.c. a marked fall in pressure. They also found that a solution of KCl in the same concentration produced a marked reduction in pressure when the same dosage was employed.

It seemed to us at the beginning fair to assume that, if potassium ions are responsible for the fall in blood pressure observed after intravenous injection of liver extract, then an aqueous solution of KH_2PO_4 or KCl containing these substances in the same concentration as they occur in the liver extract should have an equally strong depressor action.

We first analyzed a sample of liver extract with which we had been working (Heparmone of Eli Lilly & Company) and found that it had a potassium content equalling 0.9 per cent KH_2PO_4. This liver extract was tested against 0.9 per cent aqueous solutions of KH_2PO_4. As shown in Fig. 1, the liver extract, in doses of 0.1 c.c., 0.2 c.c., 0.5 c.c. and 1 c.c., showed, on intravenous injection, marked depressor effects, while a 1 per cent aqueous solution of KH_2PO_4, in the same dosage, produced no lowering of blood pressure. The 1 per cent aqueous solutions of KH_2PO_4 produced no depressor effect until a dosage of 2 c.c. was reached, the fall here being slight and corresponding in fall to that produced by 0.1 c.c. of liver extract. The liver extract then had a depressor effect equal to at least twenty times that produced by an aqueous solution of KH_2PO_4, whose content in potassium ions was equal to that of the liver extract. (Figs. 1 and 2.)

In another experiment, the total solid content of the liver extract was determined and found to be 2.494 gm. per 100 c.c. An aqueous solution of KH_2PO_4 was then prepared containing 2.494 mg. per 100 c.c. and its effect

*From the Department of Internal Medicine, the University of Kansas School of Medicine. Received for publication, November 15, 1929.

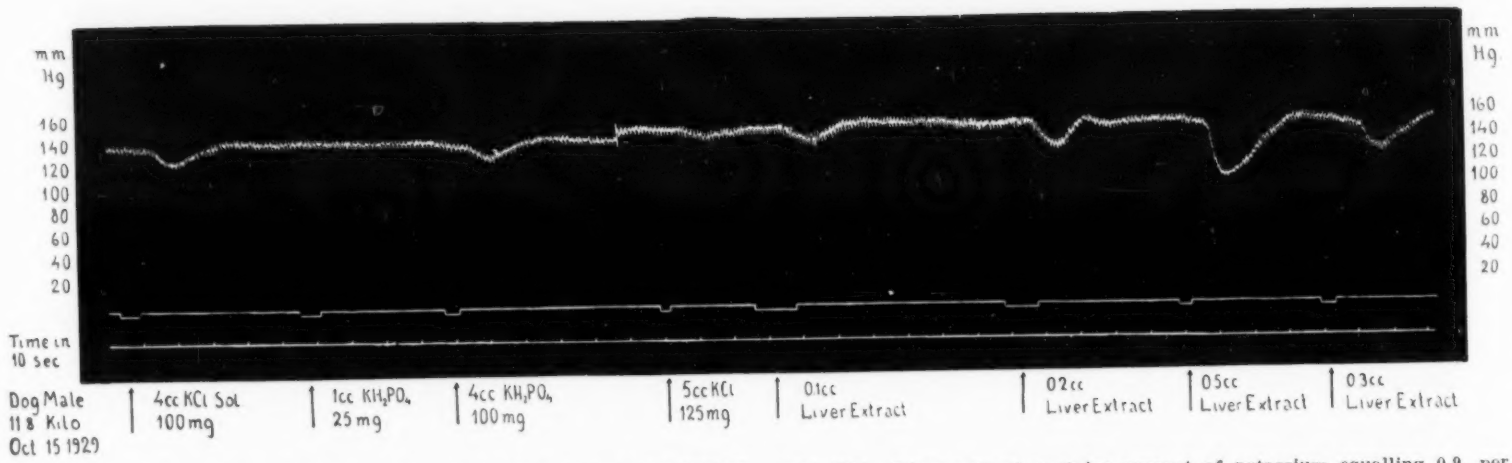

Fig. 1.—Comparison of depressor activity of liver extract with KH_2PO_4 and KCl. Liver extract contains amount of potassium equalling 0.9. per cent KH_2PO_4. Nine-tenths per cent aqueous solution of KH_2PO_4 and KCl employed. Intravenous injections.

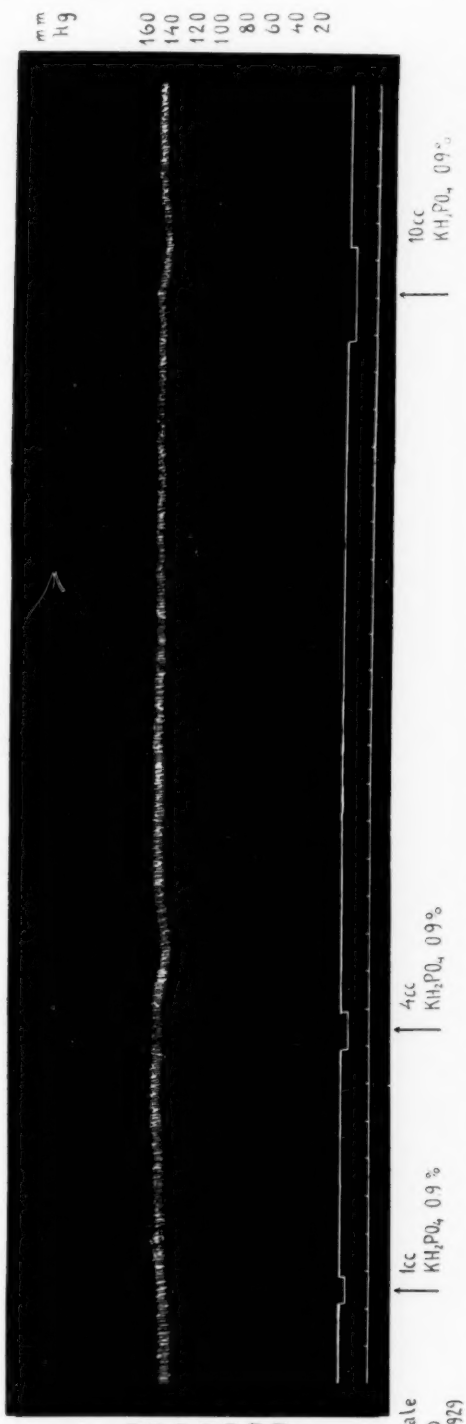

Fig. 2.—Effect of KH_2PO_4 on blood pressure. Intravenous injections. Compared with depressor activity of liver extract as shown in Figs. 1 and 3.

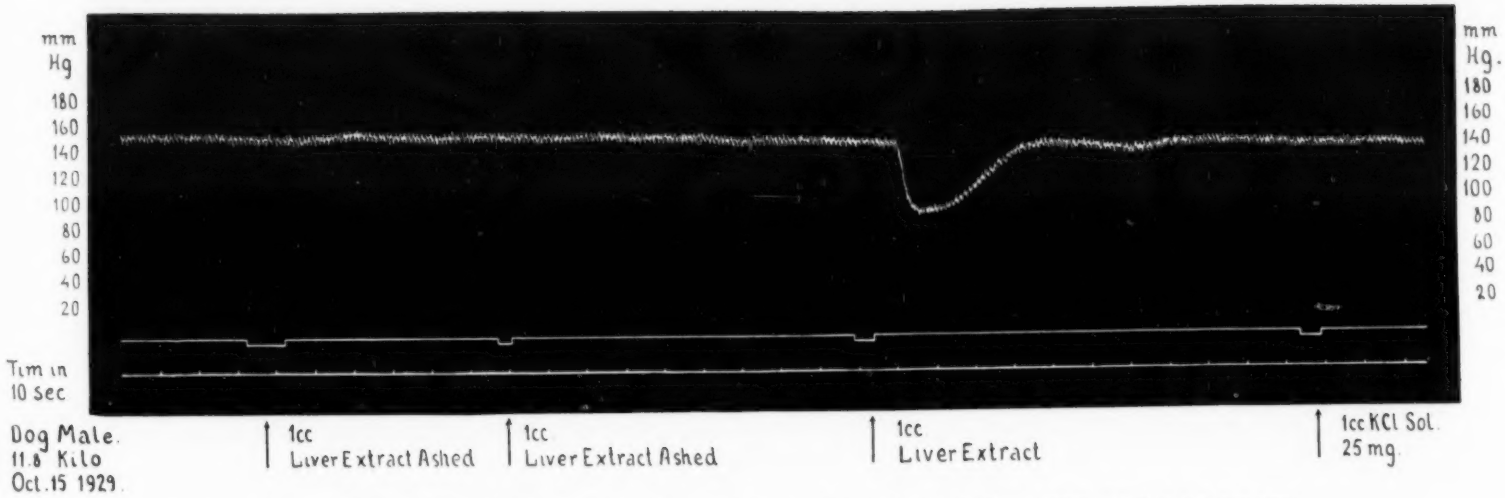

Fig. 3.—Effect of ashed liver extract on blood pressure. Intravenous injections. KCl solution 0.9 per cent.

upon the blood pressure noted. It was found, when compared with liver extract, to have an extremely feeble depressor effect. This experiment shows that if the entire solid content of the liver extract were KH_2PO_4, there is not enough present to account for its depressor effect.

We also ashed the solids of the liver extract and then redissolved the ash in distilled water, making the solution up to its original volume. These solutions had no effect upon the blood pressure, which would not have been the case if its activity were due to potassium ions. (Fig. 3.)

In most of our experiments dogs were employed, but the same results were obtained in the cat, although the cat appeared to be somewhat more sensitive to the depressor action of potassium ions.

We can confirm the observations of Goerner and Haley, that 20 per cent solution of KH_2PO_4 and KCl have a marked depressor effect upon the blood pressure of dogs and cats and may produce the death of an animal in from one to two minutes. The toxic effect of potassium ions upon the contraction of the heart is a well-known physiologic effect and in our experiments the lowering of the blood pressure produced by 20 per cent potassium solutions was accompanied by a marked enfeeblement of the heart's action and, where the blood pressure fell to zero, the heart promptly ceased beating.

CONCLUSIONS

1. The active depressor substance in the liver extract with which we have worked is not KH_2PO_4, since the liver extract in the doses employed does not contain enough potassium ions to produce the depressor effect which is obtained.

2. The fall in blood pressure obtained with 20 per cent solutions of KH_2PO_4 is due to what physiologists have long described as "potassium inhibition" of the heart and has no close analogy with the fall produced by our liver extract. Such solutions of KH_2PO_4 contain at least twenty times as much potassium as the solutions of liver extract with which we have worked.

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CONCERNING CERTAIN FACTORS WHICH MAY INFLUENCE THE SUGAR CONTENT OF THE BLOOD AND URINE*

By E. M. Watson, M.D., M.R.C.P.(Edin.), London, Canada

OF THE several factors operating to cause variations in the blood-sugar concentration and in the output of sugar in the urine, the food intake is of prime practical importance. So important is the food factor that one is prone, perhaps, to neglect the significance of those more obscure influences, such as minute changes in the intra- or extracellular acid-base equilibrium, which are known to have an important bearing upon the processes of internal or tissue respiration and in directing the activities of the tissue enzymes.

While the fluctuations which occur in the blood and urine sugar are variable, it is generally conceded that a temporary elevation of the blood-sugar concentration, accompanied by a transient glycuresis, follows meals. It would appear, however, that the extent of the increment of the blood sugar and the increase in the output of sugar is not the same following all meals of the day, even when the meals contain an equal amount of carbohydrate. For example, Page,[1] observing the hourly sugar excretion of individuals on a standard diet, found that, in general, the greatest increase seemed to follow breakfast. He noted also that the amount of sugar excreted was not entirely dependent upon the quality or quantity of the food ingested. Likewise, the daily blood-sugar curves obtained by Miller, Jonas and Teller,[2] from non-diabetic and from diabetic subjects, showed, in a considerable proportion of cases, the highest point to occur after the first meal of the day.

Those who are familiar with the practical problems related to the management of patients with diabetes mellitus, know that from the laboratory standpoint, at least, the severe diabetic is frequently at his worst in the morning. The morning glycosuria is oftentimes difficult to control, even when the carbohydrate content of the breakfast is less than that of the other meals and the morning dose of insulin is greater than the amounts given at other times of the day.

From the foregoing remarks, it would seem that there exists in many instances, an inherent tendency toward hyperglycemia and glycosuria during the early part of the day, which in the case of the individual with normal carbohydrate metabolism does not proceed to actual hyperglycemia and glycosuria but in the case of the individual with an unstable carbohydrate metabolism, the tendency is exaggerated and the findings then fall within the range of the abnormal.

The explanation usually advanced for the more marked hyperglycemia and glycosuria of diabetics following breakfast is concerned with the sup-

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posed inactivity of the glycogenic function of the tissues during the night and its delayed reactivation in the morning. To quote the words of Gray,[3] "The fasting pancreas or liver is not ready for glycogen formation." There is, as it were, a latent period during which the absorbed glucose accumulates in the peripheral blood stream but once glycogen formation becomes started, glycogenesis proceeds, more or less effectively. This is analogous to what occurs in normal persons following the repeated administration of glucose. The ingestion of glucose appears to "stimulate in some way the mechanism of carbohydrate disposal so that repeated ingestion of the same amount causes less marked hyperglycemia."[4] This lag in glycogen formation by the tissues is of undoubted importance but it is not yet decided whether it is the only factor deserving consideration when searching for an explanation for the prominence of the morning hyperglycemia and glycosuria of diabetics.

Hatlehol,[5] in the course of an extensive investigation of the changes which occur in the sugar of the blood, observed that the blood-sugar concentration of fasting diabetics fell from morning to evening with the lowest level during the night, and that it rose again during the early hours of the following day. This increase he termed the "paradoxical rise of the blood-sugar concentration." The phenomenon seemed to occur especially in the more severe cases of diabetes but it was observed in the milder ones as well and reappeared in one fasting period after another. The "paradoxical rise" was not demonstrated in healthy subjects. Hatlehol came no nearer an explanation of the problem than to suggest that it is probably related in some way to an influence on metabolism closely connected with sleep or with the waking state. He recommended that periodicity and transition from one state to another should be taken into account when judging the effects of meals or other external factors on the blood sugar.

Search for a change in metabolism associated with the transition from the sleeping to the waking state which might have a possible bearing upon the question under consideration is not very enlightening. One is reminded, however, of the change in the acid-base equilibrium described by Leathes[6] as occurring at such a time. It appears that during sleep, the respiratory center is inactive, consequently, CO_2 accumulates in the blood and a relative acidosis results. Upon awaking, arising, and resuming the duties of the day, the respiratory center becomes re-activated, CO_2 is washed out of the blood, leaving the latter with a relative excess of base. Evidence of such an occurrence is the high CO_2 content of the alveolar air immediately upon awaking and its subsequent fall during the course of the morning. Accompanying this physiologic variation in the alveolar CO_2 is a change in the reaction of the urine resulting in the so-called "morning alkaline tide" which appears to be related to the alteration in respiratory activity which occurs at this time of the day rather than to any functional activity on the part of the digestive organs.[7]

Any change in the blood of sufficient consequence to cause detectable alterations in the urine must be capable, presumably, of exerting an influence upon the delicately balanced reactions which take place within the tissues. The morning is, admittedly, a period of instability; a time when physiologic readjustments must be occurring. It is to be expected, therefore, that any

tendency to an unbalanced carbohydrate metabolism would be exaggerated at this time.

Experimental evidence concerning an association between the respiratory function and sugar metabolism is not entirely lacking. Henderson and Underhill,[8] as a result of their observations are of the opinion that "acapnia is a frequent concomitant of glycosuria or at least hyperglycemia both under clinical and experimental conditions." The experiments carried out by Imrie[9] showed that voluntary hyperpnea in normal individuals caused an increase in the blood-sugar concentration accompanying a drop in the plasma bicarbonate. While experimental conditions are often remote from those ordinarily encountered clinically, the above findings point to a probable association between the acid-base equilibrium and certain phases of carbohydrate metabolism.

With such a probability in mind, it remains to be seen if the association in question can be applied in the elucidation of the practical problem of why the diabetic is worse in the morning. The observations which form the basis of this paper were carried out upon a series of nondiabetic hospital patients and upon an equal number of diabetic patients. None of the latter were of the "severe" type. Each patient received, during the two days of the experiment, meals of equal composition. Each meal consisted of protein 10 g., fats 25 g. and carbohydrates 10 g. On the second day, breakfast was omitted. The patient emptied his bladder every two hours from 6 A.M. until 10 P.M. into bottles containing toluol. The urine secreted between 10 P.M. and 6 A.M. was collected as one sample. Samples of blood for blood-sugar estimations were withdrawn every two hours between 8 A.M. and 8 P.M. The blood-sugar estimations were carried out according to the Folin-Wu technic[10] and the urine sugar was estimated by the method of Folin and Berglund.[11] The variations in the reaction of the urine may be considered to reflect, for the purposes of the present investigation, the changes in acid-base equilibrium, as referred to above. The method used for determining the reaction of the urine was that described by Leathes.[6] The basis of this test rests in the fact that the urine reacts like a solution containing both mono- and di-basic phosphates. A measured volume of urine (10 c.c.) was diluted with two volumes of distilled water and two drops of phenolphthalein indicator and three drops of methyl orange indicator added. The mixture was then titrated with decinormal H_2SO_4 to the turning point of the methyl orange, then with decinormal NaOH to the turning point of the phenolphthalein. The result of the first titration gives the equivalent of the alkaline phosphate, while the second titration represents the total phosphate. The percentage of the total phosphate in the form of alkaline phosphate is designated the "alkalinity per cent." Calvert, Mayrs and Milroy[12] found this method to compare favorably with P_H determinations for purposes of studying alterations in the reaction of the urine.

Chart I is a diagrammatic representation, based upon the results of the above-mentioned observations, showing the changes which occur in the blood-sugar concentration, the hourly output of sugar in the urine and the urinary reaction, under the conditions of the investigation. The relationship between the three observed values is depicted. The curves of the diabetic group show

the same variations and follow the curves of the nondiabetic group but at a higher level. The events of the first day are not remarkable. There is shown the postprandial rise of the blood sugar and the accompanying increase in output of sugar in the urine. The morning alkaline tide occurs, the alkalinity per cent beginning to increase before any food has been taken. The fact that the chart shows the urine to be more alkaline in the diabetic group than in the nondiabetic group is regarded as coincidental and is not stressed. On the second day, the patients having received no breakfast, the curves tend to conform in a general way to those of the first day with the exception of the blood sugar which does not rise so high in the morning as it does at the cor-

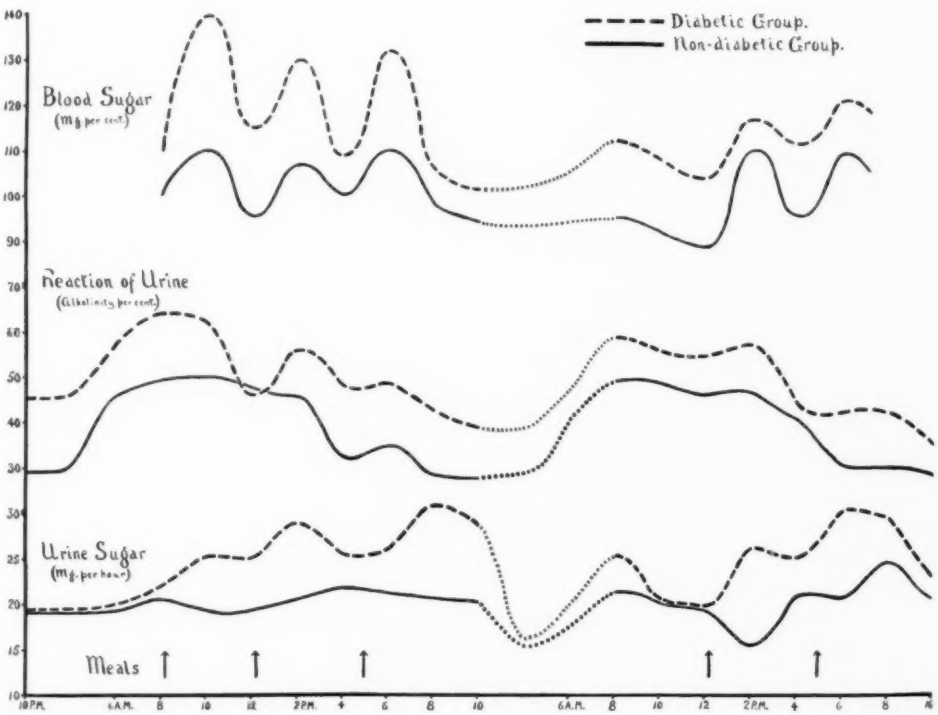

Chart I.

responding hour on the first day but there is, nevertheless, a rise, more marked in the diabetic cases. There is also an increase in the sugar excretion in the morning compared with that during the night. It will be noted that the rise in the blood sugar and the increase in the urinary sugar correspond in point of time to the increase in the alkalinity of the urine. This relationship between the three variables fails at times of the day other than the morning.

One hesitates, in the absence of more specific data, to emphasize the significance of an association between the reaction of the urine and the sugar content of the blood and urine. Benedict and Osterberg[13] noted that alkaline urines contained larger quantities of sugar than acid urines and that the transition to an acid reaction was almost invariably accompanied by a sharp fall in the rate of sugar elimination. In any case, the problem deserves fur-

ther consideration and it is possible, until the contrary is proved, that the alteration in the acid-base equilibrium which accompanies the change from the sleeping to the waking state may exert an influence upon the blood and urine sugar of diabetics if not upon normal individuals, thereby augmenting the effect produced by food.

SUMMARY

1. The relationship between the blood-sugar concentration, the hourly output of sugar in the urine, and the reaction of the urine has been studied in a series of nondiabetic and in a series of diabetic patients on a standard diet.

2. There appeared to be no constant relationship between the reaction of the urine and the sugar content of the blood and urine except perhaps during the morning.

3. When breakfast was omitted, an increase in the output of sugar in the urine occurred, and in the case of diabetic individuals, there was an obvious rise in the blood sugar as well. These increments accompanied an increase in the alkalinity of the urine.

4. Since it has been shown elsewhere that the morning alkaline tide of the urine appears to be related to an alteration in the acid-base balance associated with the change from the sleeping to the waking state, it is suggested that the same process of readjustment may be a factor in causing an increase of the blood and urine sugar during the early part of the day.

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STUDIES IN THE PHARMACOLOGY OF LOCAL ANESTHETICS*

II. Irritation as Produced by Local Anesthetics on Rabbit's Cornea

By Harold W. Coles, Ph.D., and C. L. Rose, Indianapolis, Ind.

COCAINE has long led the field of local anesthetic compounds, as far as the efficiency in topical application has been concerned; but because of its extreme toxicity many substitutes have been proposed and tried. Some of these have been successful. Others have failed because their properties did not include the necessary effect when applied topically to mucous membranes. The assaying of these many compounds has entailed an immense amount of experimentation upon absorptive membranes, involving chiefly the cornea of the rabbit.[1, 2, 3, 4] The greater part of these tests has been concerned primarily with the duration of anesthesia and has overlooked the accompanying degree of irritation, a consideration that is of great importance in evaluating a local anesthetic substance. This may have been due in part to the difficulty that such an experiment always presents in the way of recording. The photographic plate offers possibilities that have not been utilized in this field.

A simple method has been devised for recording irritation as evinced by inflammation of ocular and palpebral conjunctiva, and pitting and mucus formation on the cornea and sclera. A diagrammatic drawing is made representing the conjunctiva and cornea. This is filled in with markings and color of varying degrees of intensity, to indicate the range of irritation, and filed as part of a permanent record of a given local anesthetic.

Irritation has been defined as an exaggerated response to stimuli, but it must be more closely limited to make that definition apply to the rabbit cornea method.[1] Irritation may be, but does not necessarily have to be accompanied by pain, by engorgement of the capillaries (incipient inflammation) of the cornea and conjunctiva, or by a temporary but none the less severe corrosion of the superficial cell layers over the iris and pupil. In response to pain the eye will be tightly closed, the lids being pressed together and wrinkled. Capillary engorgement is easily recognized by an increase in the intensity of the coloring of the palpebral conjunctiva and by an added redness on the sclera. Unfortunately, it will be found more often than not, that the anesthetic showing the longer duration will also show a greater corrosive action on the cornea. The latter results in a temporary softening of the superficial cell layers over the iris, producing pits and cracks. Shreds of mucus may appear also. These conditions are recorded, and upon their degree of presence or absence depends the value of local anesthetic compounds as far as irritation is concerned.

As a rule, an individual under the identical conditions of dose and metab-

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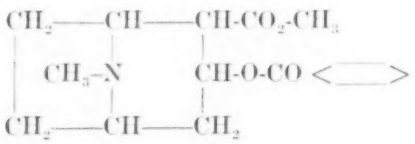
olism will always respond in such a way as to give an exact number of minutes' duration or a definite degree of irritation. But all individuals do not react in the same manner, and for the purposes of a physiologic experiment it is customary and best to employ a group of animals. For this particular type of work it was found that about 10 per cent of the rabbits used in the original groups gave durations that were as much as fifteen to thirty minutes longer or shorter than the other 90 per cent. The irregular 10 per cent were discarded. This left a group of animals which was constant in response under given conditions, and which might be said to be standardized by selection.

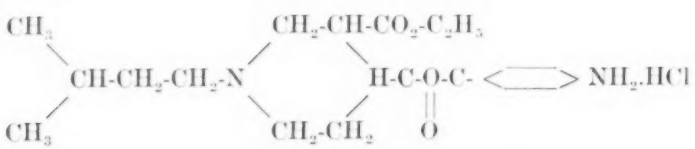
Following the method of Schmitz and Loevenhart, three rabbits in apparent good health were selected for each group, and isolated. The long hair and lashes were closely clipped from about their eyes, and pinching the lower lid to form a cup, 15 drops of a 2 per cent anesthetic solution were instilled into each eye. The solution was allowed to remain there for one minute and then was drained out. The eye was observed before instillation and a record made of its appearance in the normal state; then, after the local anesthetic had attained its maximum effect, the eye was again examined by the aid of an ophthalmoscope and a record made of its changed condition. In order that any disturbance occurring on the cornea might be attributed to the local anesthetic alone, a glass rod with a dull rounded end was used to stimulate the cornea when determining duration.

Twelve preparations have been selected to show the variations obtained by the instillation of different substances into the conjunctival sac of the normal rabbit eye. Plate I represents the degrees of irritation produced on the rabbit cornea and conjunctiva by

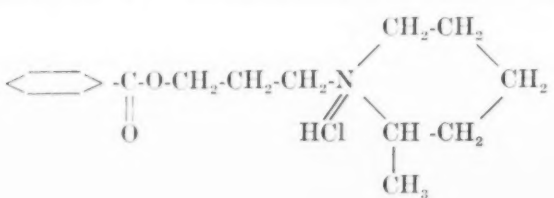
Cocaine,



(5) #0 1-isoamyl-3-carbethoxy-4-piperidyl p-amino benzoate HCl,



(6) #33G Gamma-2-(methyl piperidino)-propyl benzoate HCl,



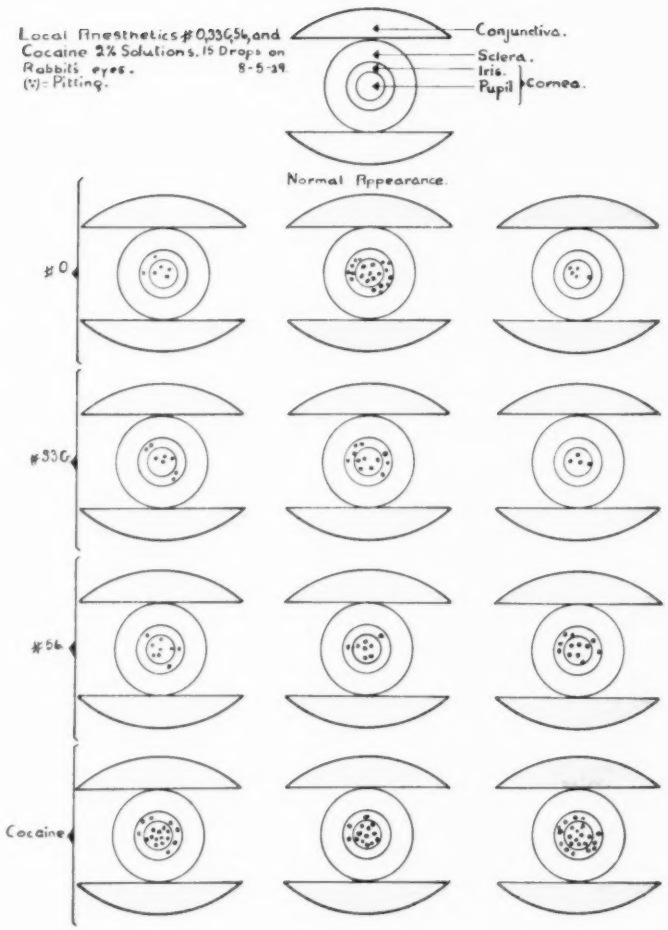

Local Anesthetics #0, 336, 56, and Cocaine 2% Solutions. 15 Drops on Rabbit's eyes. 8-5-29.
(x) = Pitting.
Conjunctiva.
Sclera.
Iris.
Pupil
Cornea.
Normal Appearance.
#0
#336
#56
Cocaine

Plate I.

(7) and #56 Gamma-(3-methyl piperidino)-alpha methyl propyl benzoate HCl,

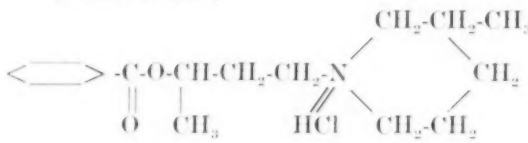
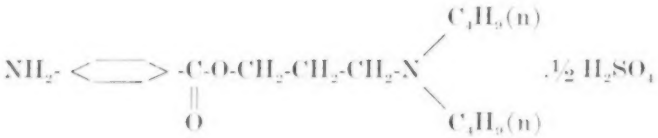
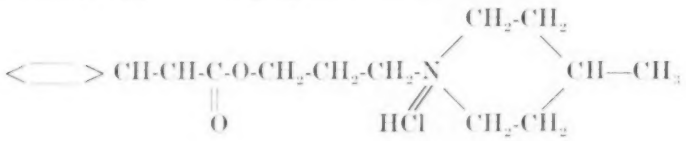


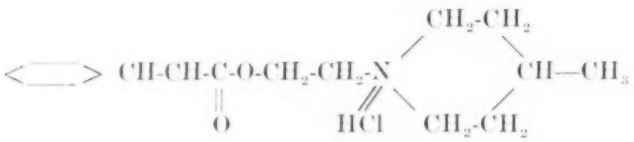
Plate II is a continuation of Plate I and represents the irritation caused by butyn,



*#93 4-methyl piperidino propyl cinnamate HCl,



*#92 4-methyl piperidino ethyl cinnamate HCl,



(8) and #58 1-phenyl ethyl-4-piperidyl p-amino benzoate HCl,

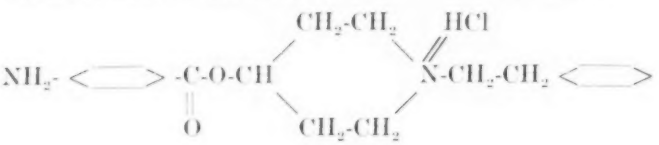
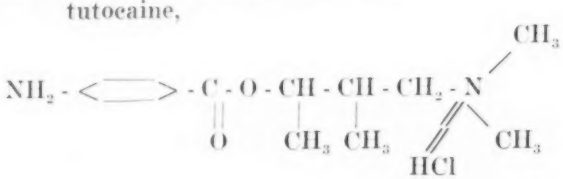


Plate III shows the results obtained from the use of distilled water, normal physiologic saline, Corydalis tuberosa "B," and tutocaine,



From the accompanying plates it may be seen that each of the compounds tested has certain characteristics which produce the reactions seen in the eyes to which it has been applied.

*The paper describing the chemistry of these compounds, which were obtained from Professor S. M. McElvain of the University of Wisconsin, for the purpose of physiologic testing, is in print and will be published by him in the *Journal of the American Chemical Society.*

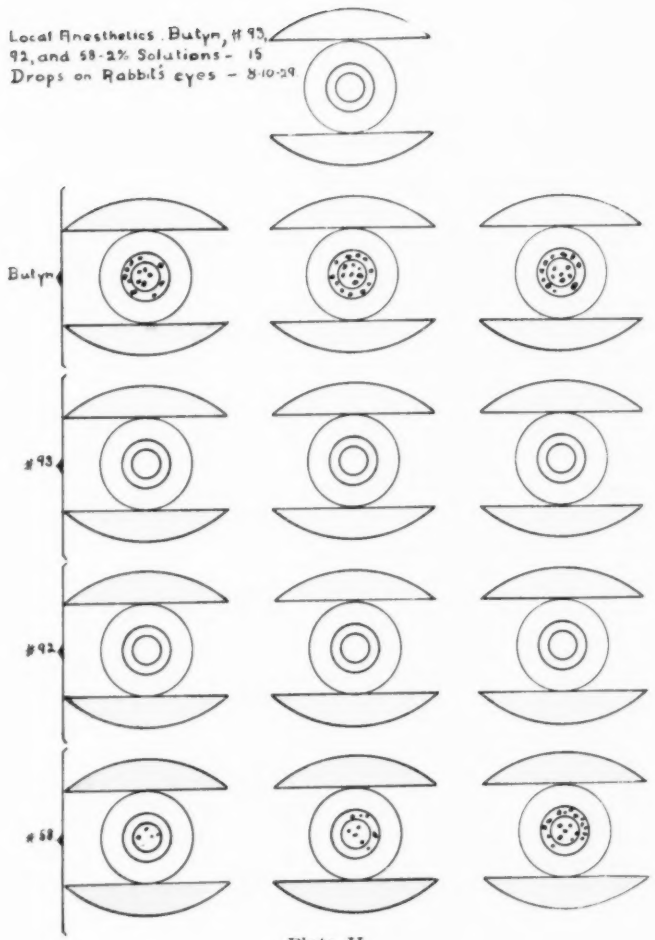

Local Anesthetics. Butyn, # 93, 92, and 58-2% Solutions - 15 Drops on Rabbit's eyes - 8-10-29
Butyn
93
92
58

Plate II.

By grouping the substances whose characteristics are most alike, they may be evaluated as follows:

Group I.—Very slight engorgement and no pitting
Distilled water
Normal physiologic saline

Group II.—Slight engorgement and slight pitting
Tutocaine
#33
#56

Group III.—Slight engorgement and severe pitting
Cocaine*
#0

Group IV.—Severe engorgement and severe pitting
Butyn
#58

Group V.—Very severe engorgement and little or no pitting
#93
#92
Corydalis tuberosa "B"

Extent of irritation, as defined in this paper, is not correlated, apparently, with the duration of anesthesia. Corydalis tuberosa "B," #93 and #92 show severe engorgement but are effective for only sixteen minutes or less. On the other hand, all those compounds producing more or less severe pitting are responsible for considerable duration. This is shown in the accompanying table.

COMPARISON OF PITTING AND DURATION OF ANESTHESIA

INSTILLATION MATERIAL	PITTING	DURATION
Distilled water	negative	negative
Normal physiologic saline	"	"
#93	"	13 minutes
#92	"	16 "
Corydalis tuberosa "B"	"	15 "
Butyn	3 plus	30 "
#33	1 "	40 "
#56	1 "	40 "
Tutocaine	1 "	40 "
#0	1 "	60 "
#58	1 "	60 "
Cocaine	1 "	60 "

CONCLUSION

1. Irritation produced by local anesthetics on the rabbit's cornea is given a limited definition in order that the term may apply specifically to the particular conditions here encountered.

2. A method for the permanent recording of irritation phenomena on the rabbit's cornea is described.

3. Irritation, in extent, is not paralleled by duration of anesthesia, although pitting is accompanied (with the materials studied), by a prolonged

*Cocaine possessing the property of being astringent to mucous membranes shows less engorgement than might otherwise be expected.

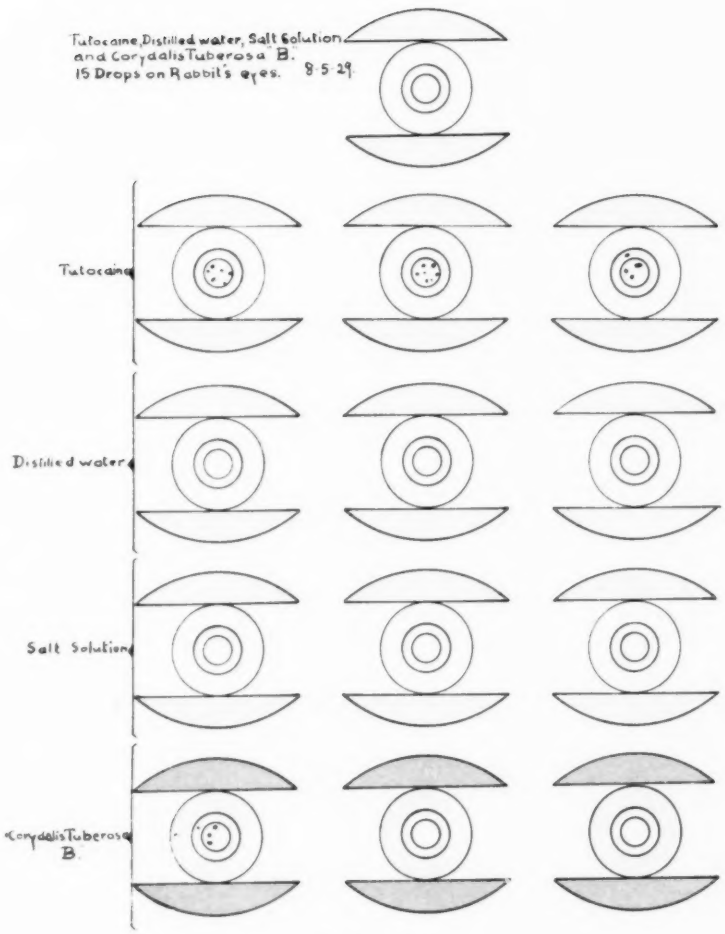

Tutocaine, Distilled water, Salt Solution and Corydalis Tuberosa B.
15 Drops on Rabbit's eyes. 8-5-29
Tutocaine
Distilled water
Salt Solution
Corydalis Tuberosa B.

Plate III.

anesthesia. This may, however, show reversals, such as mild pitting and long anesthesia, as against very severe pitting with anesthesia of shorter duration.

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LABORATORY METHODS

THE ESTIMATION OF HEMOGLOBIN. A NEW HEMOGLOBINOMETER*

By Francis F. Schwentker, B.S., M.D., Baltimore, Md.

THERE is probably no single laboratory procedure of greater importance than the estimation of hemoglobin and on the other hand, probably none less satisfactory. The remedy for this condition has been eagerly sought, as is evidenced by the flood of articles which has swept the literature since the introduction of the first clinical hemoglobinometer by Gowers. However, a satisfactory method seems never to have been developed. Those procedures giving results accurate to 1 per cent to 2 per cent require quantities of blood available only by vena puncture. On the other hand, all those methods for which sufficient blood may be procured from a finger prick, show errors of 5 per cent even when the most scrupulous care is exercised and constant vigilance is maintained regarding the standard solutions.

HISTORICAL

Ten different principles have been employed for the estimation of the hemoglobin content of blood. These may be enumerated as follows:

1. Direct color comparison method
2. Acid hematin method
3. Carbon monoxide method
4. Iron content method
5. Oxygen capacity method
6. Specific gravity method
7. Spectroscopic method
8. Spectrophotometric method
9. Refractometric method
10. Extinctometric method

Since the literature is peculiarly lacking in any review of the large number of articles which have been published on this subject, an analysis of the work is included here.

Direct Color Comparison Method.—This principle was first introduced by Gowers (1878) who diluted a measured volume of blood until its color quality matched that of a standard picrocarmine solution. The dilution necessary gave a measure of the hemoglobin content of the blood. Von Fleischel later introduced a wedge shaped standard which in turn was modified to the form of the modern Fleischel-Meischer instrument in which blood diluted to a given volume is compared in color to a standard wedge. This instrument is so calibrated that the hemoglobin content may be read directly from the wedge in grams of hemoglobin per 100 c.c. of blood.

*From the Laboratory of Physiological Chemistry, the Johns Hopkins University.
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Bürker (1924, 1925) adapted this principle to the Dubosque colorimeter so that the color of diluted blood might be compared to that of a standard. The standard used consists of a sealed constant-depth chamber which is introduced into the colorimeter below the prism.

In 1900 Tallqvist devised the simple procedure of blotting up a drop of blood on a piece of filter paper and comparing the color with a series of standard lithographed shades. The color values descend in 10 per cent gradations from a maximum 100 per cent which is indicated as the color of normal blood containing 5,000,000 red blood cells per c.mm. This was later modified so that the color scale formed the circumference of a disc which might be enclosed in a watch-like case and so turned that any desired color value appeared at a small window (Roderick, 1926).

The Dare instrument (Dare, 1900) enjoys the most widespread use of any in this group. A drop of undiluted blood is drawn into the capillary space between two small plates of glass. One of these is milk glass, the other transparent. The plates are slipped into a groove in the instrument and the color of the blood compared through an eyepiece with that of a standard disc which may be so rotated as to give any desired color value.

The objection that shades of red are difficult to match colorimetrically hangs over all the instruments in this group and has caused their general supersedence by others of greater accuracy. The Tallqvist is probably the least reliable of the series, being basically little better than a crude estimation. The Dare instrument, however, seems to have gained considerable popularity in spite of its cost and the fact that it has many times been shown to have an accuracy little greater than the Tallqvist (Lindsay, et al., 1926; Mills, 1925; Osgood, 1926; Senty, 1923). In fact the general error of the instruments constructed on this principle has been shown by different observers to range from 10 per cent to 40 per cent. Osgood (1926), Mills (1925), Lindsay (1926) and others feel that the Dare hemoglobinometer gives readings which are accurate to only 20 per cent to 29 per cent. A number of other observers (Christensen, 1924; Lilliendahl-Petersen, 1914; Lindell, 1899; Lucey, 1923; Osgood, 1926; Senty, 1923) have found the Tallqvist method susceptible to errors ranging from 2 per cent to 40 per cent. In addition, Lucey has pointed out that the picrocarmine standard employed by Gowers fades on standing and that any slight acidity of the water used as a diluent causes a change in color quality.

Acid Hematin Method.—Ever since the introduction of this method by Sahli it has been the target for the majority of criticisms and modifications in the literature. Although this is a fair indication of the dissatisfaction it has afforded, it also marks this process as the most adaptable so that it has continued to find favor with clinicians in general.

The hemoglobin in the blood is converted to the brown acid hematin by the addition of 0.1 N hydrochloric acid and the color produced compared to a standard. The Lilliendahl-Petersen method (1914) utilizes as a standard a series of strips of paper of varying shades of brown. The blood is blotted up on a bit of filter paper which has previously been impregnated with a hydrochloric acid solution and its color value determined by comparison with the standard shades. Obviously, this, too, is a crude estimation although some

workers have reported it to be more accurate than the Tallqvist (Christensen, 1924).

The acid hematin principle has also been adapted for use with the ordinary types of laboratory colorimeter. In such a case the blood is diluted after the production of the acid hematin and compared in color to a standard solution. This is usually prepared from a quantity of normal blood containing 5,000,000 red blood cells per c.mm. Plesch (1910), Autenrieth and Konigsberger (1910), and Haden (1922) have recommended the prism type of colorimeter (Hellige) as less expensive, although the regular Dubosque plunger type instrument is usually employed since it forms a necessary part of every laboratory equipment (Cohen and Smith, 1919).

The original apparatus of this type was devised by Sahli (1895). It consists of a small graduated tube in which the blood and acid are mixed. When the brown color of the acid hematin appears, the solution is diluted by the addition of water until its color matches that of a similar standard solution in a sealed glass tube. The level of the liquid in the graduated tube gives a measure of the hemoglobin content of the blood.

Haessler and Newcomer (1916) and later Trimble (1922) modified this method so that the blood is diluted to a definite volume and its color then matched with one of a series of 10 standard tubes representing intervals of 10 per cent of the normal.

There are several disadvantages and sources of error which creep into this method. Chief among these is the fact that the color of the standard solutions fades rapidly, even though kept in hermetically sealed tubes. This has been definitely proved by a number of workers (Lindsay, et al., 1926; Terrill, 1922; Felton, 1923) and although the rate of fading is quite inconstant it may average a 50 per cent loss of color in eighteen months. Many substitutes have been suggested for the standard acid hematin solution. Terrill (1922) uses a concentrated solution of acid hematin prepared by evaporating ordinary preparations, preserving with glycerol and sealing in ampules. This is diluted for standards as required and according to this author loses 8 per cent of its color value in ten months. The same investigator has prepared a dried powder which may be weighed out accurately and dissolved to form a standard acid hematin solution. The stability of such a powder is somewhat greater than that of concentrated solutions kept in ampules. Several authors have advocated more simple substitutes giving the brown color of acid hematin. Felton (1923) suggests the alkaline oxidation product of pyrogallic acid; Jacobson (1919) prefers rufigallic acid solutions (hexaoxyanthrachinon). A German investigator (Lipp-Weingarten, 1918) advocates a solution of henna, while a standard prepared from ferric and chromium sulphates has also been suggested (Haskins, 1923; Osgood and Haskins, 1923). Standards of colored glass rods have been produced for the Sahli instrument (Leitz and Hellige, 1924) but the color match is difficult. Newcomer (1919, 1923) has introduced as a standard a plate of brown semaphore glass into one side of a colorimeter. Osgood and Haskins (1923) and Robscheit (1920) consider the color in this modification too pale to allow of accurate determinations and it has been found that variations in the temperature of the

solution cause perceptible errors. However, there are others who have welcomed this innovation.

The second great disadvantage in this method is the delay in the formation of the maximum color of the acid hematin when the blood and acid are mixed. It has been shown that the rate of formation of the color in an acid hematin solution follows a rectangular parabolic curve of the form $x = -ay^2$ and that the percentage error in the final reading at any moment is equal to $40/m$ where m denotes the number of minutes between mixing the blood and acid and taking the reading. Thus it may be seen that in order to produce a reading with an intrinsic accuracy to 1 per cent, forty minutes must be allowed to elapse before the reading is taken. This work has been corroborated by Meulengracht (1916, 1921) and Komiya and Katakura (1922). Using this formula Newcomer (1923) has devised a table by which the reading at any time may be corrected to a maximum value.

Several workers have suggested heating the mixture in the water-bath to hasten the color formation. Berman (1919) advocates boiling for one minute. Osgood and Haskins (1923) advise a temperature of 55° to 60° C. for seven minutes. Komiya and Katakura (1922) have shown that the maximum color formation occurs in five minutes if the solution is heated at 60° C., in ten minutes if at 50° C., and in fifteen minutes if 30° C. is maintained. They feel, however, and are supported by Terrill (1922) that a cloudiness is produced by temperatures over 30° C., so that the color match is thus rendered more difficult. These same authors suggest that the reading be taken in one minute and the instrument so standardized as to give the correct values at this time. They have shown that the color formation in such a process runs parallel to the true hemoglobin value.

Several minor difficulties are also experienced. Differences of 1 mm. in the bore of Sahli tubes have been shown to introduce an error of 25 per cent to 33 per cent unless the instrument is individually standardized (Kuttner, 1916). Staubli (1911) has shown that great care must be exercised in adding the correct amount of acid since the final reading is increased by only a slight excess of the acid. Terrill (1922) feels that the standard solutions tend to become turbid on standing only two months. Berezeller (1918) has stated that variations in the lipoid content of the blood will alter the readings appreciably. And finally errors in pipettes, etc., obviously affect the results, but such errors are inexcusable in any well-regulated laboratory. Several slight modifications intended to facilitate estimations have also been described (Thro, 1925; Thisted, 1925; Kuttner, 1915).

The acid hematin method has been demonstrated under some conditions to give results showing errors which vary among the different observers from 5 per cent to 40 per cent (Berman, 1919; Christensen, 1924; Haden, 1926; Lindsay, et al., 1926; Müller, 1925; Osgood, 1926; Robscheit, 1920; Lebermann, 1925). Notwithstanding, it is generally agreed that when carefully undertaken with instruments which have been scrupulously standardized and whose standards have been checked within a month, results are obtainable with errors within 5 per cent to 7 per cent.

The Carbon Monoxide Method.—In 1892 Hoppe-Seyler described a method for the determination of the hemoglobin content of the blood by saturating a known dilution of the blood with carbon monoxide. This converts the hemoglobin into carbon monoxide hemoglobin whose color is compared to a known standard. It proved too complicated for general clinical use until Haldane (1900) adapted it for use with the Gower colorimeter. Later Palmer (1917, 1918) modified the method for laboratory analysis to the Dubosque type of colorimeter. Kämmerer and Schaulin (1924) suggested a series of standard tubes in a comparison rack such as is used for P_H determinations. In each case the carbon monoxide is supplied by bubbling coal gas through the mixture.

This method has found no great favor as a clinical process. Coal gas is not always available and, although Miller (1923) has shown that acetylene may be used instead, the method is somewhat cumbersome. Robscheit (1920) and Appleton (1918) state that the standard solutions deteriorate rapidly and must be renewed every month. Lucey (1923) has shown that the water for dilution must be slightly alkaline to litmus to prevent flocculation. If ordinary coal gas is used as a source of carbon monoxide a N/200 solution of potassium hydroxide is employed instead to neutralize the acidity caused by solution of the gas. On the whole, this method gives results accurate to 5 per cent if the standards are fresh and the determinations carefully made.

Iron Content Method.—Jacquet (1894), Hüfner (1878, 1894), and Butterfield (1909) have shown that the iron content of hemoglobin is 0.334 per cent of its total weight. Since then, Wong (1923), Berman (1919), and Fowweather (1926) have devised colorimetric methods for the quantitative estimation of iron in blood. These methods are too time consuming and require too great an amount of blood for routine clinical work, but their accuracy is rather high (1 per cent) and they have thus found favor as a means of standardizing less accurate clinical instruments.

Oxygen Capacity Method.—In this method the oxygen capacity of the blood is determined as a measure of its hemoglobin content since it has been shown that 1 gm. of hemoglobin combines with 1.34 c.c. of oxygen (Hüfner, 1894). To Haldane and Smith (1899) goes the credit for the first reliable method of measuring the oxygen capacity but Van Slyke (1917, 1918, 1921, 1924) later modified the process so that it has become available for ordinary laboratory work.

Although no information has been given by Van Slyke as to the accuracy of his first instrument, reference is made to the work of Lundsgaard (1918) who shows, in four series of duplicate determinations, intrinsic errors of 0.14 per cent, 3.0 per cent, 1.4 per cent and 0.7 per cent respectively. Later Van Slyke and Stadie (1921) published the results of duplicate analyses on a later modification of the original instrument. These show a maximum deviation of 2.1 per cent. Since then, however, an entirely new instrument has been devised which, although more complex, shows a maximum variation in analysis results of 0.48 per cent (Van Slyke and Mill, 1924). Its accuracy seems to have gained this process a place in modern methods of blood analysis, notwithstanding the fact that considerable experience and time are essential for trustworthy analyses.

Specific Gravity Method.—A method for estimating the hemoglobin content of blood has been described by Lindell (1899) who accredits the process to Hammerschlag. This technic has been devised on the theory that the hemoglobin content and the specific gravity of blood have a definite relation. Since this assumption has been proved to be incorrect by the data of Meyer and Butterfield (1914) and Newham (1924), the method will not be considered further.

Spectroscopic Method.—This principle is one of the oldest employed for the determination of the hemoglobin content of blood. Preyer (1866), Quincke (1872), and Rajewsky (1876) have compared the spectrum of diluted blood with that of a known solution of hemoglobin. Sahli (1895) has described the Henoch hematoscope in which a solution of blood in a wedge-shaped container is viewed through a spectroscope, the depth of the solution being adjusted by movement of the wedge until the two bands of oxyhemoglobin in the yellow portion of the spectrum are equal in width. Finally, Henri and Wurmser (1912) have devised a method in which the blood is diluted until the absorption band in the green portion of the spectrum disappears. Each of these methods, however, seems to have been short-lived; particularly since the large type of spectroscope employed rendered the apparatus bulky and expensive.

Spectrophotometric, Refractometric and Extinctometric Methods.—These last three principles may be quickly dismissed from this discussion since they are seldom made use of in clinical determinations. The spectrophotometer compares the light value of the spectrum of an unknown hemoglobin solution with that of a standard (Martens and Grünbaum, 1903). The refractometer is occasionally used to standardize less accurate clinical instruments (Stoddard and Adair, 1923; Gram, 1925; Howard, 1920). The refraction afforded a beam of light by an unknown solution of hemoglobin is compared to the refraction produced by a standard solution. The least adaptable of the three is the extinctometer (Wolvius, 1924). In this instrument the absorption of light by a solution of unknown hemoglobin content is measured by the intensity of the transmitted rays and compared to known standards. Each of these methods, while obviously accurate, requires an operating time and a technical knowledge too great to allow of general use.

DESCRIPTION OF AUTHOR'S METHOD

A method has been devised by the author which makes use of the spectroscopic principle and which shows an accuracy comparing favorably with any existing technic. A discussion of its principles and procedures follows.

Theoretical.—Oxyhemoglobin is one of the few compounds whose spectrum shows a marked variation in the number and width of the absorption bands with changes in the concentration of the solution. This was first observed by Hoppe-Seyler (1862, 1864) and later by Stokes (1864). Rollet in 1880 undertook a quantitative study of these changes and has produced a series of curves plotting the variations in the absorption bands with concentration of the solution under analysis. Fig. 1 is a reproduction of the curve for oxyhemoglobin for a fluid depth of 1 cm. To ascertain the amount of absorption for any given concentration up to 1 per cent, a horizontal line may

be drawn across the diagram at the level corresponding to the concentration. Where this line passes through the shaded part of the diagram, absorption takes place, and the width of the absorption bands is seen at once. The diagram clearly shows that as the concentration is increased three bands become apparent, two in the yellow portion of the spectrum between the D and E lines and one in the extreme blue. These become wider until at a concentration between 0.6 per cent and 0.7 per cent the two bands in the yellow portion fuse and for a greater concentration, between 0.8 per cent and 0.9 per cent, they are joined by a band in the blue.

Beer in 1852 showed that there exists for any solution a simple relation between the absorption, the concentration and the depth of the solution under analysis. This may be expressed by the equation

$$-\log T_\lambda = k_\lambda \, cd \qquad \text{(Equation 1)}$$

where T is the light transmittance for the particular wave-length, c is the concentration of the solution, d represents the thickness of the fluid stratum and k_λ is a constant for the working wave-length.

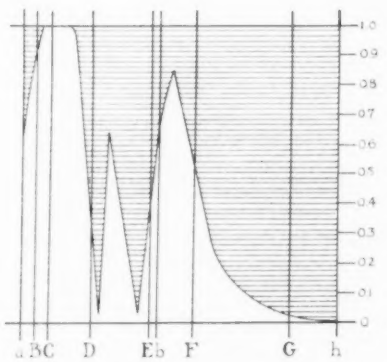

Fig. 1.—Diagram to show the variations in the absorption spectrum of oxyhemoglobin with varying concentrations of the solution (After Rollet). The numbers to the right give the strength of the oxyhemoglobin solution in percentages; the letters give the positions of the Frauenhofer lines. To ascertain the amount of absorption for any given concentration up to 1 per cent, a horizontal line may be drawn across the diagram at the level corresponding to the concentration. Where this line passes through the shaded part of the diagram absorption takes place, and the width of the absorption bands is seen at once.

For the conditions of this experiment, in which the transmittance is a constant and attention is limited only to a single portion of the spectral field, the equation becomes

$$cd = A \qquad \text{(Equation 2)}$$

where A is an absorption constant.

Applying this to the foregoing, it is evident that absorption is proportional to the concentration as well as to the fluid depth so that the same changes in the absorption bands may be elicited by variations in the depth of the fluid stratum as are caused by changes in the concentration.

It was shown by Reid (1905) that oxyhemoglobin forms a true solution and may thus be reasonably considered to follow this same law. Some time later Butterfield (1912) was able to prove that blood itself in dilutions from 0 to 200 acts as a true solution and follows this relation between absorption, fluid depth, and concentration.

The idea immediately presents itself that the oxyhemoglobin content of blood may be determined by means of this equation. If blood be diluted to a known volume and the depth of the fluid stratum varied until the spectrum caused by it assumes some predetermined picture, the concentration of the oxyhemoglobin in the solution will be inversely proportional to the known depth. Or

$$c = A \frac{D}{d} \quad \text{(Equation 3)}$$

where c = the concentration of oxyhemoglobin in the blood (gm. per 100 c.c.)
A = the absorption constant (determined experimentally)
D = the dilution (c.c.)
d = the depth of the fluid stratum (cm.)

This principle has been employed by the author to determine the hemoglobin content of blood, since the oxyhemoglobin and hemoglobin contents are

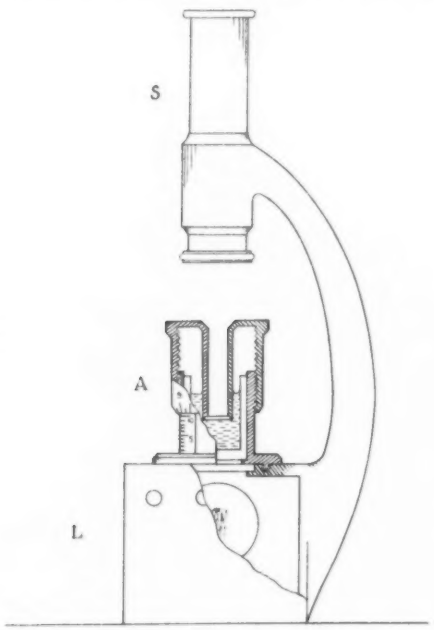

Fig. 2.—Sketch of the author's instrument. A direct vision hand spectroscope, *S*, is so mounted that the spectrum of the fluid in the absorption chamber, *A*, may be viewed through it. Illumination is furnished by a small 20 watt microscope lamp enclosed in a case at *L*. The completed instrument is furnished by E. Leitz, Inc., 60 East Tenth Street, New York City.

obviously interlinked. The fluid stratum of blood, diluted to a known volume, is varied until the two absorption bands between the D and E lines are just separated. The hemoglobin content is then estimated from the foregoing equation (Equation 3), the constant *A* having been determined experimentally.

Description of the Instrument.—Fig. 2 is a sketch showing the assembly of the instrument devised by me for estimating the hemoglobin content of blood. A small direct vision spectroscope, *S*, is so mounted that the spectrum of the fluid in the absorption chamber, *A*, may be viewed through it. The variation in the fluid depth is produced by screwing down or up the cap of

the plunger. Readings are made from the micrometer scale on the side of the instrument. It has been found essential to have a fixed source of light, *L*, since variations in the illumination, such as would be incident with ordinary daylight, cause considerable errors in the readings.

The Method.—In this procedure 0.1 c.c.* of blood is added to 3 c.c. of water and the two mixed by stirring. Laking is almost instantaneous in this dilution. This fluid is placed into the absorption chamber of the instrument and its spectrum viewed through the spectroscope. The plunger of the ab-

Fig. 3.—Photograph of hemoglobinometer used by the author.

sorption chamber is screwed down until a fine line of yellow light just appears between the two absorption bands. At this point the reading of the fluid depth is taken and from it the hemoglobin content calculated by means of the following relation derived from Equation 3.

$$c = 31 \frac{A}{d} \quad \text{(Equation 4)}$$

Experimental.—Obviously this necessitates the determination of the constant A of the foregoing equation. Since variations in the light have been

*Since the completion of this article, the amount of blood required has been reduced from 0.1 c.c. to 0.025 c.c. by reducing the size of the absorption chamber.

shown to affect the hemoglobin readings, this constant should be determined for each individual instrument. This may be accomplished by the analysis of bloods or solutions of known hemoglobin content, with subsequent substitution of the results in Equation 4. It must be borne in mind, however, that in such a case the constant has an accuracy no greater than that of the method employed for the determination of the hemoglobin content of the standard blood.

The constant for the author's instrument having been determined, a group of experiments was undertaken to ascertain the accuracy of the process. Solutions of known hemoglobin value were prepared from weighed amounts of pure crystalline oxyhemoglobin and analyzed by this method. Table I shows how close is the agreement. Even on bloods of low hemoglobin content the readings check within 0.7 per cent which is an accuracy obtainable with few clinical hemoglobinometers. In fact methods not generally used clinically, which require bloods in amounts from 2 c.c. to 5 c.c. give results no better than this.

Experiments were then undertaken to ascertain the effect of jaundice upon the hemoglobin estimation by this method. It is well known that the results of any of the colorimetric methods are unreliable in the presence of bilirubin in the blood serum. Varying amounts of bile obtained through can-

TABLE I

ACCURACY OF ANALYSES

HEMOGLOBIN CONTENT GM. PER 100 C.C.		DIFFERENCE
GRAVIMETRIC	SPECTROSCOPIC	PER CENT
20.032	20.09	0.24
15.024	14.95	0.49
10.016	10.06	0.44
7.512	7.46	0.70

nulation of the common duct of an anesthetized dog were added to measured quantities of blood. Equal amounts of physiologic saline were added to another series as controls and the hemoglobin contents of both groups analyzed. Table II gives the results. It is seen that bile present in amounts up to 20 per cent of the total volume of the blood has no effect upon the accuracy of this method. This is a distinct advantage since heretofore accurate determinations on jaundiced patients have been possible only by using some method other than colorimetric; all of these requiring more blood than can be procured from a finger prick.

In addition, such an instrument is useful for the qualitative analysis of such substances as hemoglobin in the urine or elsewhere, for methemoglobin, carbon monoxide hemoglobin, etc. Methods for the quantitative analysis of these last two substances are being developed.

There are several criticisms which might arise from a consideration of this method. For, quite obviously, if the hemoglobin of the blood were not totally saturated with oxygen so that it had been entirely converted to oxyhemoglobin, considerable errors would be introduced. It has been found, however, that enough oxygen is absorbed by the blood during its exposure on the finger tip and from the water with which it is diluted to cause no appreci-

able error in the results. On the other hand, the oxygen dissolved in 3 c.c. of water is more than sufficient to oxidize the reduced hemoglobin of the blood of even cyanotic patients.

TABLE II

EFFECT OF JAUNDICED BLOOD ON HEMOGLOBIN ANALYSES

PER CENT BILE IN BLOOD	HEMOGLOBIN ANALYSIS RESULTS GM. PER 100 C.C.		PER CENT DIFFERENCE
	JAUNDICED BLOOD	CONTROL BLOOD	
4.77	13.38	13.50	0.90
9.10	12.96	13.00	0.31
20.00	11.21	11.15	0.54

Finally it would seem that any changes in the temperature of the blood solution would cause errors in the results of the analyses. However, the ordinary variations in room temperature have been found to introduce no perceptible change in the hemoglobin reading. From a theoretical standpoint the difference in fluid density resulting from a change of 10° C. in the solution temperature would cause a variation in the final result of only 0.1 per cent, which is well within the instrumental error.

CONCLUSIONS

This spectroscopic method for the determination of the hemoglobin content of blood has the following advantages:

1. It is simple, requiring no technical knowledge.
2. Its error is less than 0.7 per cent.
3. It is rapid, a complete analysis requiring approximately three minutes.
4. It requires no chemicals, solutions or colorimetric standards which are liable to fading and require replacement.
5. It requires only as much blood as can be drawn from the finger tip (0.1 c.c.).
6. Its accuracy is unaffected by the presence of bile pigments in the blood.
7. It may be used for all spectroscopic qualitative tests.

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A MICRO-MODIFICATION OF B. GRUSKIN'S DETERMINATION OF UREA IN BLOOD*

By Henry Tauber, Ph.D., and Boris Kwartin, M.D., Brooklyn, N. Y.

A METHOD for the estimation of urea in blood has been worked out by B. Gruskin,[1] using 1 c.c. of oxalated blood in his method. We modified this method by using 0.2 c.c. of blood which is drawn from the finger tip in the usual manner.

PRINCIPLE

Urea is decomposed to ammonium carbonate by means of urease; the proteins are precipitated, and the urea is determined colorimetrically by direct nesslerization.

REAGENTS

1. *Jack bean urease solution.* (Folin.)

2. *A precipitation mixture* used for the preparation of the protein-free filtrate is made up in the following manner: Use 7 parts of distilled water, 1 part of a 10 per cent sodium tungstate solution, and 1 part of $\frac{2}{3}$ N sulphuric acid. Shake thoroughly.

3. *Nessler's reagent.* (Koch-McMeekin.)

4. *Stock ammonium sulphate solution:*

Ammonium sulphate, C. P.	0.4716	gm.
Concentrated hydrochloric acid	1.0	c.c.
Water, ad q. s.	1000.0	c.c.

Micro standard is made by taking 10 c.c. of this solution and diluting it to 100 c.c. One hundred c.c. of the micro standard equals 1 milligram nitrogen

The ammonium sulphate should be dried in hot air for one-half hour at 110° C.

METHOD OF PROCEDURE

Two-tenths c.c. of oxalated blood is placed in a specially constructed, ground glass stoppered, 15 c.c. graduate centrifuge tube, containing 1 drop of a 1.2 per cent potassium oxalate solution, and shaken. Add 0.2 c.c. of the jack bean urease solution and place in a water-bath of 45° to 50° C. for ten to fifteen minutes. One and six-tenths c.c. of the precipitation mixture are added; the tube is stoppered and inverted several times. Let stand 3 minutes. Centrifuge for two or three minutes at high speed. Pipette off 1 c.c. of the clear, colorless supernatant fluid and place in a 5 c.c. graduate centrifuge tube. Add 2 c.c. of distilled water and then 0.6 c.c. of Nessler's reagent. Dilute to mark 5 and read in the colorimeter against the standard solution, which is set at 20. For standard, take 1.5 c.c. of the micro standard; 2 c.c. of distilled water, and 1.5 c.c. of Nessler's reagent in the order given.†

*From the Biochemical Laboratory, Beth Moses Hospital, Brooklyn, N. Y.

†If the urea is very high, it is advisable to dilute the supernatant fluid.

CALCULATION

$\frac{\text{Standard}}{\text{Reading}}$ times 15 equals mg. urea per 100 c.c. of blood.

COMMENT

The method is very simple and easily applied; the technical procedures are reduced to a minimum. The use of the precipitation mixture in place of the separate reagents is of great advantage. The calculation is the same as in the macromethod because the relative proportions remain the same. Numerous parallel determinations from oxalated venous blood and capillary blood obtained by finger puncture show the differences in the reading so minute as to be negligible.

COMPARATIVE VALUES OF MACRO- AND MICROMETHODS

SPECIMEN NO.	UREA MG. PER 100 C.C. OF BLOOD		DIFFERENCES
	MACRO	MICRO	
1	15.2	15.2	0
2	14.25	14.5	0.5
3	15.6	16.1	0.5
4	13.95	13.25	0.70
5	15.15	14.5	0.65
6	13.5	15.2	1.7
7	50.0	54.0	4.0
8	39.1	40.2	1.1
9	26.8	25.6	1.2
10	8.6	9.3	0.7

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A SIMPLE CENTRAL CATALYZING STATION TO PRODUCE ANAEROBIOSIS*†

By John Cohen, M.D., New York, N. Y.

THE most successful methods thus far discovered for the production of strict anaerobiosis for bacteriologic purposes, make use of the catalytic combination of hydrogen with the last traces of oxygen left in a partially evacuated system. Several devices using this principle are described in the literature, but many of them are either only partially successful or extremely expensive.

In this paper a simple and inexpensive apparatus is described which gives excellent results.

The apparatus shown in Fig. 1 consists of the following parts:

A is a low pressure hydrogen tank and *B* is a reducing valve. To the latter there is attached a pressure tube, *C*, which in turn is attached to a glass tube, *D*. This *D* is an inlet tube to a mercury manometer, *E*, and is connected with the latter through a glass stopcock, *F*. The other end of the glass tube, *D*, is connected by means of a pressure tube, *G*, to a central catalyzing station, *H*. The central catalyzing station consists of a brass tube, 60 cm. in length and 2 cm. in diameter with walls 3-4 mm. in thickness. Twenty cm. from the connection between the pressure tube, *G*, and brass tube, *H*, there is a connecting brass tube, *J*, 10 cm. in length, 1 cm. in diameter with a 2 mm. wall which is brazed into tube *H* (see cross-section diagram). Side tube *J* is connected by means of pressure tubing, *O*, to a source of vacuum. Through the opening, *K*, of brass tube *H* there is inserted a roll of finely meshed copper wire containing palladinized asbestos. The copper wire should be made to fit the inside of the tube snugly but not too tightly, so as to allow free passage of hydrogen. The copper wire mesh is inserted up to the brazed end of connecting tube *J*. Over the opening, *K*, of brass tube *H* there is attached a pressure tube, *L*, which in turn is connected by means of a short glass tube, *M*, to another length of pressure tube, *N*. The latter is slipped over the nozzle, *P*, of the top of a specially constructed jar, *Q*. The jar, *Q*‡, is of glass, 15 cm. in diameter and 28 cm. in height, which is closed by an aluminum cover, *X*, which in turn is clamped into place by clamp *R*. The latter is fed through an aluminum stand, *S*. Different sized jars, of course, may be used for this apparatus. The joint between the cover and the jar is sealed with a high grade modeling putty softened with castor oil. All the other joints are tightened with copper wire. The rubber tubes are painted with castor oil. Two Hoffman clamps, *T* and *U*, are placed over the connecting rubber tubes, *C* and *N*, respectively.

*From the Laboratories of Mount Sinai Hospital, New York.

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‡The jar with aluminum covers and stands can be obtained from the Empire Laboratory Supply Company, New York City.

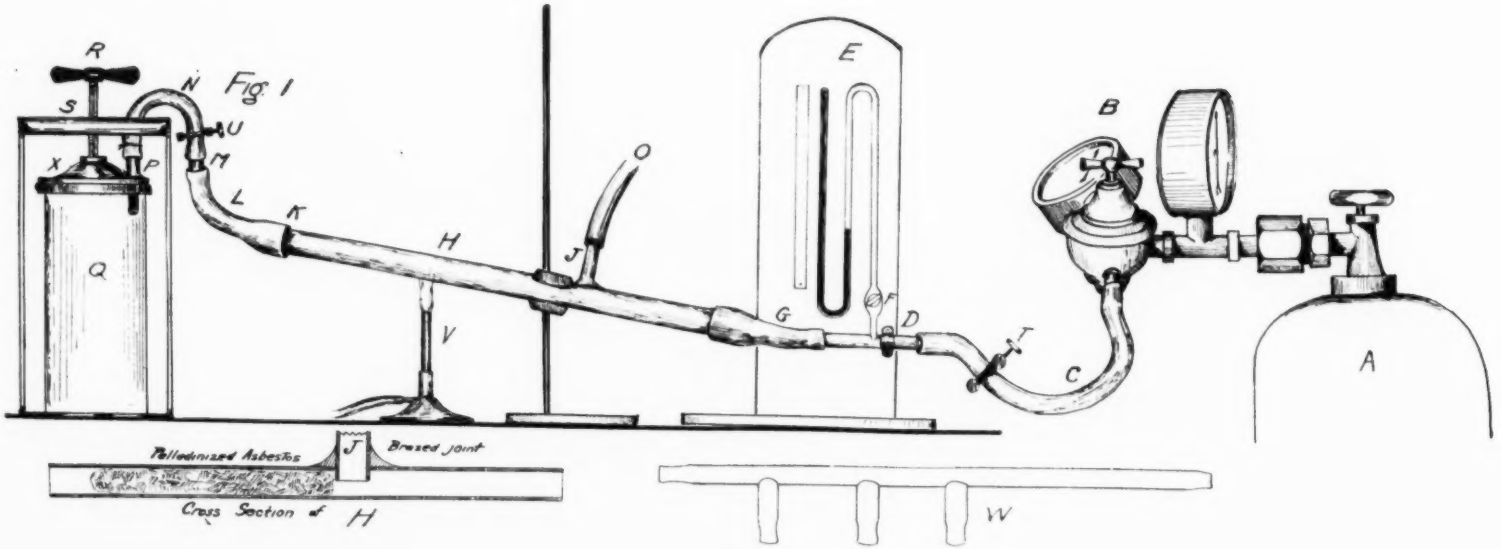
Fig. 1
R
S
N
U
M
X
P
Q
L
K
H
O
J
E
B
G
D
F
I
C
A
V
W
Palladinized Asbestos
J
Brazed joint
Cross Section of H

Fig. 1.

OPERATION OF APPARATUS

Tighten clamp *T;* open clamp *U;* open stopcock *F;* evacuate the system through pressure tube *O* until there is a drop in the mercury manometer to about 20 mm. While the evacuation is taking place, the cover, *X*, settles firmly on the jar, *Q;* tighten clamp *R* further and remold the putty on the seam between cover and jar. Shut off the vacuum at *O*. Watch the mercury manometer to detect any leak in the apparatus. Heat the brass tube, *H*, to a dull red heat by means of a Bunsen burner, *V* (better still a blast lamp). Open clamp *T*. Open the hydrogen tank and allow the gas to flow at a slow rate. Stop the flow of hydrogen when the mercury in the manometer returns to atmospheric pressure. After a minute or so open the hydrogen tank again and allow a few more cubic centimeters of hydrogen to flow into the jar. Shut off the hydrogen tank and tighten clamp *T*. After two or three minutes tighten clamp *U*. The jar, *Q*, and stand, *S*, may now be removed with the connecting tube, *N*, and the clamp, *U*, attached.

Instead of the connecting tube, *M*, a glass manifold, *W*, may be substituted and in this manner several jars may be rendered anaerobic at the same time.

The palladinized asbestos in tube *H* can be periodically examined by forcing air through *O*. This expels the copper mesh with the palladinized asbestos through *K*.

As a test for anaerobiosis in the jar, a tube containing 10 c.c. of 1 per cent glucose broth colored with 0.1 c.c. of 1 per cent aqueous methylene blue is placed in the jar. The liquid should become completely decolorized in a few hours in the incubator at 37° C.

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A SUGGESTION FOR COLLODION SACS*

By Miles J. Breuer, M.D., Lincoln, Nebraska

THERE are certain clinical laboratory methods depending on the use of a collodion sac or capsule, which are not used as widely as they ought to be, because of the difficulty in making these sacs successfully. The research man who spends some months on a problem in which these sacs are required and during which time he needs large numbers of them, may develop the skill and

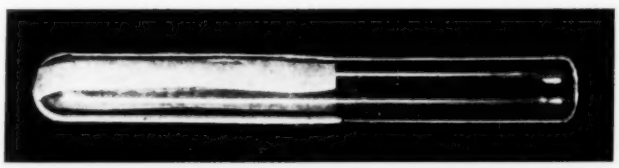

Fig. 1.

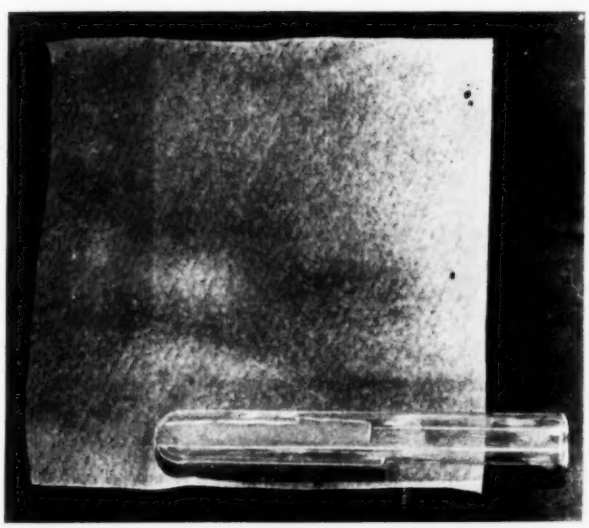

Fig. 2.

facility necessary to make them successfully. The man engaged in clinical practice, who needs a sac only occasionally, finds great difficulty in getting constant and dependable results in making collodion sacs.

I have talked with a number of men in charge of clinical laboratories, and find that the reason that they are not interested in such procedures as the dialysis method for the P_H value of the blood or the Abderhalden reaction, is that they are unable to make the sacs. After finding my own efforts to make

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these sacs most uncertain, I corresponded with a number of laboratory men, but was quite unable to get any real assistance or information on the subject. Those who are successful in making these sacs, consider it easy and cannot understand why some one else cannot make them; and yet they do not seem to be able to get their technic down on paper.

In casting about for some method for securing dependability of results in making these sacs, I hit upon the expedient of using a paper support for the collodion. This method, with a slight amount of practice, works uniformly, and gives a strong, rigid sac, much easier to handle than the unsupported kind.

There is a good deal of literature on the subject of making collodion sacs, but everything I have been able to find concerns itself with the *permeability* of the collodion membrane, and therefore with the composition of the collodion mixture entering into its making, and with the methods of drying, hardening, etc. This paper does not concern itself in any way with the question of per-

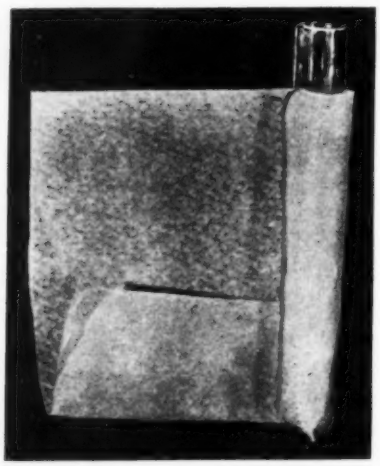

Fig. 3.

meability, but merely with a support for any type or kind of collodion membrane that is necessary for any particular procedure. All the modifications of collodion mixture and all the variations of permeability are quite applicable in this method, for the tissue paper support is in no way concerned with the permeability of the sac. It merely acts as a coarse framework to support the collodion membrane.

The method is extremely simple and is far easier to carry out than to explain. Use a small test tube as a mold on which to form the wet tissue paper support. The tube in which commercial Loeffler's coagulated blood serum for diphtheria cultures is purchased makes a convenient size, being about 0.75 by 9 cm. A thin toilet paper makes a satisfactory paper.

In order to make the bottom of the paper capsule tight so that the collodion will not leak, it is necessary to lay three strips of the tissue paper, about 0.5 by 10 cm. over the bottom of the tube, as shown in Fig. 1. Wet the strips and they will adhere to the tube and remain in place. In the illustration only

two strips are shown for the sake of clearness; a third strip should be put on, and all of the glass surface covered.

Then lay the tube on a piece of damp tissue paper, allowing an inch to project over at the bottom, as in Fig. 2. Roll one turn about the tube, and then fold the projecting portion back on the tube, as in Fig. 3. The rolling is then finished, and the tube with the paper on it, as in Fig. 4, is allowed to dry. After it is dry the glass is readily removed and we have a paper capsule, Fig. 5.

Fig. 4.

Fig. 5.

Now, follow the procedure usually used in making the collodion sac in a glass tube. Fill the paper form with collodion for a few seconds, empty, and drain upside down for one to three minutes. If the mouth of the tube shows a tendency to become too hard and dry, dip into collodion for a centimeter of its length, while the upper portion is setting. After it has set for not over three minutes, repeat. Usually this is done three times, but of course here we are entering the domain of permeability, which has no business in this article. After the collodion has set for the last time, the sac is placed in distilled water, where it is kept until needed.

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A MODIFIED VARNEY JAR FOR THE CULTIVATION OF ANAEROBES BY MEANS OF PHOSPHORUS*

By S. R. Damon, Ph.D., Baltimore, Md.

BEGINNING with the work of Sellards,[1] stick phosphorus has been used by several workers as a means of reducing oxygen tension in closed containers in which anaerobes were to be cultivated. In an attempt to simplify the technic and employ apparatus easily obtained by any laboratory, Bushnell[2] introduced the use of a pressure cooker or fruit jars in place of Sellards' more elaborate container. As an improvement over this, Varney[3] suggested the use of the ordinary museum jar five by twelve inches in size with a removable wire rack which would contain the tubes or Petri dishes to be incubated. In this laboratory this apparatus has been successfully employed in the cultivation of anaerobic spore-forming bacteria and spirochetes from the mouth.

The use of the Varney apparatus has not been unaccompanied by difficulties, however. In some instances the rubber gasket has not formed a perfect seal, and although the phosphorus ignited and was consumed, it was found that leakage occurred and we did not actually have anaerobic conditions. Our chief difficulty and expense, though, has been with breakage of the jars. In the hands of students this was not uncommon and led us to the design of a galvanized iron can that could be substituted for the museum jar. At the same time we incorporated features that enabled us to use it not only for anaerobiosis but for the cultivation of organisms favored in their growth by an increased CO_2 tension.

The apparatus is illustrated in Fig. 1. At the left is shown the museum jar as described by Varney with its internal heavy wire rack supporting the inverted tin cylinder which protects the cultures from the deposit of phosphorus pentoxide. On top of this is an asbestos ring supporting an evaporating dish and surrounded by a wire gauze screen which protects the sides of the jar from spattering phosphorus during combustion. This figure is included to show the internal assembly which is not shown in our galvanized iron container shown in the center illustration. The essential features of this container are that it is made of No. 26 gauge galvanized iron with a tightly fitting lid of the same material which overlaps the cylinder about one and a quarter inches. Obviously a lid of this construction will not be air-tight and modeling clay is used over the joint to render it so. This is quickly applied and smoothed out after the lid is placed in position and the seal is generally formed before the phosphorus ignites.

Two features of the lid should be emphasized. In the first place it is made with a projecting flange at the top, as shown in the illustration. This

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affords a grip that is an aid in removal. In the second place it has two air-tight stopcocks soldered into it. These are useful in connecting the apparatus to a source of CO_2 and a graduated cylinder, immersed in water, when it is to be used in the cultivation of organisms favored by an increased CO_2 content in their environment. Under such circumstances when the stopcocks *A* and *B* are opened and *C* closed, after submergence of the graduated cylinder in water, the entrance of CO_2 through *A* displaces air through *B* and the proportion desired may be obtained by measuring the amount collected in the cylinder.

For details of construction of the wire rack and tin cylinder reference should be made to Varney's[3] paper, as to give the instructions here would be mere repetition. Suffice it to say then that when constructed to fit a galvan-

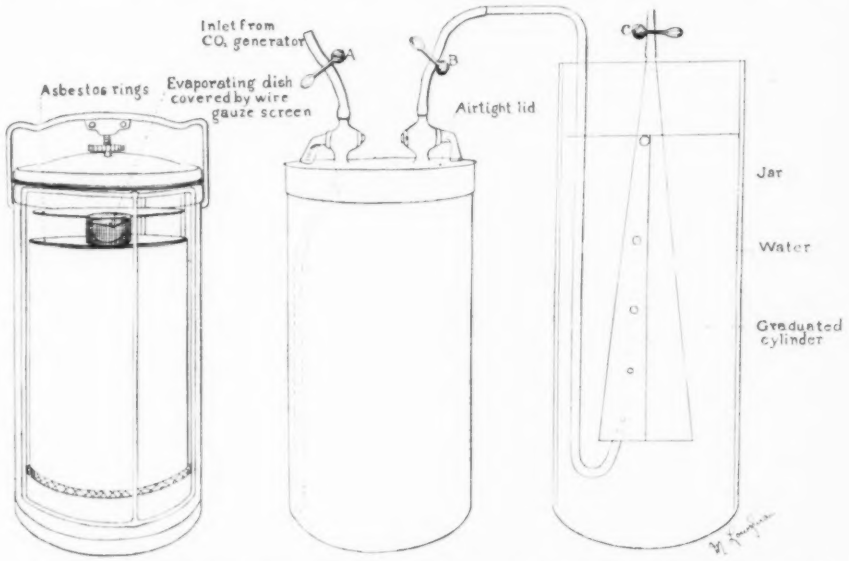

Fig. 1.—The left-hand figure represents the complete assembly of the Varney jar. The center figure shows the details of construction of the galvanized iron container with suggested connections. The right-hand figure indicates the method of collecting displaced air when an increased CO_2 atmosphere is desired in the jar containing the culture.

ized iron can six and one-half by twelve inches we habitually place twelve to fifteen plates in each container.

In the use of the apparatus the usual precautions in handling phosphorus should be observed to prevent premature combustion and burns. It may also be said that we have found it advantageous to place the whole apparatus in the ice box for a short period before opening it after it has been in the incubator. This chilling allows more leeway in the process of opening it up before combustion of the residual phosphorus.

Briefly stated, the steps in the assembly of this apparatus are:

1. Place a little water in the galvanized iron jar.
2. Load the Petri dishes or tubes on the wire rack.
3. Place the tin cylinder over the cultures.
4. Put the rack of cultures in the galvanized iron jar.

5. Place the asbestos ring on top of the tin cylinder and put the evaporating dish in place.

6. Put the piece of phosphorus in the evaporating dish and cover with the wire screen.

7. Close the stopcocks in the jar lid and place it in position.

8. Complete the air-tight seal with modeling clay.

9. Place jar at desired incubation temperature.

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THE APPLICATION OF THE PROCESS OF CIRCULATORY SOLUTION TO THE PARAFFIN INFILTRATION OF TISSUES*

By William F. Sheridan, A.B., Washington, D. C.

THE infiltration of tissues with paraffin can be facilitated by the application of the process of circulatory solution.

The process is effected by suspending cleared pieces of tissue upon a porous diaphragm near the surface of melted paraffin. The portions of the clearing reagent coming into immediate contact with the melted paraffin are diluted and distributed, and descend, their places being supplied by fresh portions of melted paraffin. A circulation is created and dilution and distribution of the clearing reagent throughout the paraffin facilitated.

The clearing reagent being miscible in the melted paraffin is displaced and the interstices of the tissues filled with paraffin containing varying amounts of clearing reagent, depending in extent upon the proportion of paraffin used in effecting the infiltration. Complete or nearly complete replacement of the clearing reagent within the interstices of the tissues can better be effected by using large volumes of melted paraffin. This will result in greater dilution of the displaced clearing reagent, and its subsequent removal from the melted paraffin will be expedited by exposing a greater surface to the air.

The paraffin bath as generally practiced: one, two, or three changes in shallow containers of very small capacity, with the tissues resting on the bottoms of the containers, obviously cannot result in complete replacement of the clearing reagent in the optimum period of time. Dilution of the displaced clearing reagent by its distribution throughout the melted paraffin is not accomplished. During part of the time considered necessary to complete the infiltration, the tissues are bathed in paraffin that contains an excessive amount of clearing reagent at the bottom of the containers and which is incapable of

*From the School of Medicine, George Washington University, Washington, D. C.
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dilution and distribution throughout the too small volume of paraffin, due (particularly chloroform s.g. 1.490) to the difference in density.

The apparatus illustrated in this paper has, over a period of two years, given results that were very satisfactory. It is inexpensive, and correct in principle for the establishment of circulatory solution. The pan, measuring 24 cm. long, 14 cm. wide and 6.5 cm. deep, is filled with 1000 gm. of paraffin, which when melted brings the surface within 2 cm. of the top of the pan. The

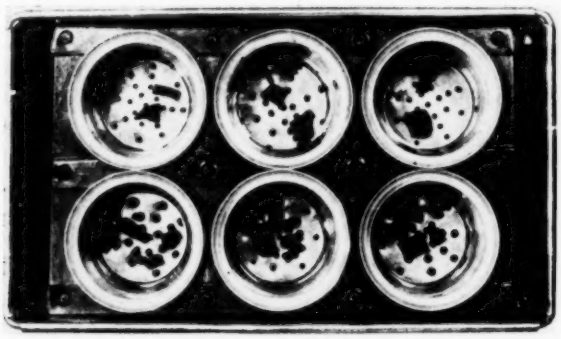

Fig. 1.

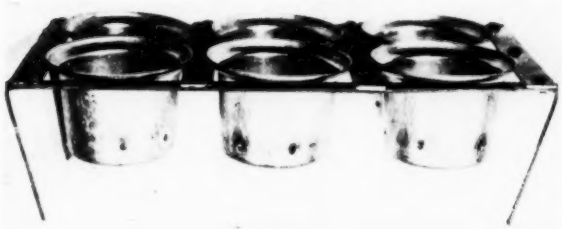

Fig. 2.

rack is made of brass 1.2 cm. wide by 0.1 cm. thick. Two strips 34 cm., one strip 22 cm. and four strips 12.5 cm. long, are bent and riveted together so that the whole conforms to the inside dimensions of the pan, furnishing six spaces each measuring 6.3 cm. by 4.5 cm. Six aluminum cups with shoulders, measuring 6.3 cm. across the top, 4.4 cm. across the bottom, and 4.4 cm. deep, with numerous small perforations on the bottoms and sides are suspended in the six spaces of the rack. The whole is then placed in an oven or other heating appliance that will maintain a temperature at or near the melting point of the paraffin.

WASSERMANN TEST WITH GLYCERINATED HUMAN SERUM*

By E. Henry Ruediger, M.D., Hollywood, California

ALTHOUGH the different steps of the Wassermann test with glycerinated human serum (Ruediger's modification of the Wassermann test) have been described previously, a concise description of the entire method seems to be in order, because the others are scattered over a period of several years.

Shortly after having begun to do the Wassermann test, I saw the importance of preserving the human serum to be sent to distant laboratories, to be used as control serums and in order to establish a unit of measure which may be used in the standardization of the Wassermann test.

Upon trying a number of different preservatives, I[1] found glycerol to be quite satisfactory for the purpose. Glycerol renders the serum moderately anticomplementary, which must be overcome by increasing the quantity of hemolytic amboceptor. The titration of hemolytic amboceptor must be done in the presence of as much glycerol as is used in performing the test.

Early in 1916, I[2] showed that glycerinated human serum keeps well for several months. In other reports, I[3, 4, 5] showed that glycerinated human serum holds its titer fairly well, provided it is kept at low temperature, preferably below zero Fahrenheit. Dried[6] human serum holds its titer much better.

ANTIGEN

In my studies on antigens,[7, 8, 9] I found alcoholic extracts of syphilitic human heart muscle and alcoholic extract of normal beef heart muscle most satisfactory. A small quantity of cholesterol, 25 mg. per 100 c.c. of extract, may be added to advantage. The antigen is diluted slowly to give an opalescent solution, and is used in the optimum dilution. In order to obtain uniform results, uniform methods must be used. In diluting the antigen, I proceed as follows: Put 0.1 c.c. of extract (antigen) into a 25 c.c. graduated cylinder;

TABLE I

ANTIGEN TITRATION

ANTIGEN DILUTION	NEGATIVE CONTROL SERUM PRECISION METHOD							POSITIVE CONTROL SERUM PRECISION METHOD						
	READINGS						RESULTS	READINGS						RESULTS UNITS PER C.C.
	ANTIGEN TUBES			CONTROL TUBES				ANTIGEN TUBES			CONTROL TUBES			
	1	2	3	1′	2′	3′		1	2	3	1′	2′	3′	
1:20	0	0	+	0	0	±	Anti-comp.	0	±	+	0	0	±	1.0
1:30	0	0	±	0	0	±	O K	0	+	+	0	0	±	1.5
1:40	0	0	±	0	0	±	O K	0	+	+	0	0	±	1.5
1:60	0	0	±	0	0	±	O K	±	+	+	0	0	±	2.0
1:80	0	0	±	0	0	±	O K	±	+	+	0	0	±	2.0
1:120	0	0	±	0	0	±	O K	±	+	+	0	0	±	2.0
1:160	0	0	±	0	0	±	O K	0	+	+	0	0	±	1.5

Explanation: 0 means complement is not fixed, or hemolysis; + means complement is fixed, or no hemolysis; ±, at least 50 per cent fixation; TR, less than 50 per cent fixation.

*From the Hollywood Clinical Laboratory.

add 0.1 c.c. of physiologic salt solution, and shake, do this twenty times. Then add twenty portions of 0.2 c.c. each, shaking after each addition of 0.2 c.c. Add portions of 0.4 c.c. of physiologic salt solution until the total quantity measures 8 c.c. The optimum dilution must be determined for each antigen. I determine the optimum dilution by titration as is shown in Table I.

According to the results shown in Table I, 1:80, I would accept as the optimum dilution.

COMPLEMENT SERUM

Complement serums[10] from different guinea pigs do not always give identical results. Therefore, I select the complement serums. Each serum is titrated against the negative control serum and against the positive control serum, as is shown in Table II. Satisfactory serums are used and unsatisfactory serums are discarded.

Table II shows three good serums and three poor serums. Serums Nos. 1, 4 and 6 do not give positive results with the Wassermann test, but show good fixation in the presence of syphilitic human serum. Serum No. 2 has poor hemolytic power, Serum No. 3 gives a positive result with the Wassermann test, and Serum No. 5 shows poor fixation in the presence of syphilitic human serum. Serums Nos. 2, 3 and 5 are rejected, and Serums Nos. 1, 4 and 6 are used in the test.

The satisfactory serums are pooled and preserved. Complement serum can be kept frozen[11] or salted.[12] I prefer to add 720 mg. of sodium chloride per 20 c.c. of guinea pig serum, and keep it at a temperature of about 1° C. For use, serum so salted is diluted 1:5 with distilled water and any further diluting is done with physiologic salt solution (0.9 per cent).

TABLE II

COMPLEMENT TITRATION

NO. OF SERUM	NEGATIVE CONTROL SERUM PRECISION METHOD							POSITIVE CONTROL SERUM PRECISION METHOD						
	READINGS						RESULTS	READINGS						RESULTS
	ANTIGEN TUBES			CONTROL TUBES				ANTIGEN TUBES			CONTROL TUBES			
	1	2	3	1′	2′	3′		1	2	3	1′	2′	3′	
1	0	0	±	0	0	±	good	±	+	+	0	0	±	good
2	0	±	+	0	±	+	poor	+	+	+	0	±	+	poor
3	0	+	+	0	0	±	bad	+	+	+	0	0	±	?
4	0	0	±	0	0	±	good	+	+	+	0	0	±	good
5	0	0	±	0	0	±	good	0	±	+	0	0	±	poor
6	0	0	±	0	0	±	good	+	+	+	0	0	±	good

PRIMARY INCUBATION

Fixation of complement in the Wassermann test is not instantaneous. Elsewhere,[13, 14, 15] I reported that fixation of complement is not always completed in five or six hours, and that the most suitable temperature seems to be near the freezing point, about 1° C.

RED BLOOD CORPUSCLES

As red blood corpuscles in the hemolytic system, I use those of man, because it is more convenient. The corpuscles are washed three or four times

with physiologic salt solution, and a 1:40 (2.5 per cent) suspension of the corpuscles is made in physiologic salt solution. The last centrifuging is done for ten minutes at fairly high speed to pack the corpuscles well.

HEMOLYTIC AMBOCEPTOR

As hemolytic amboceptor, I use the blood serum of rabbits that have been immunized against human blood corpuscles, which have been well washed with physiologic salt solution. The rabbit serum is heated to about 56° C. for thirty minutes, and is mixed with an equal volume of glycerol No. 1. In the precision method and in the routine method described elsewhere,[16] I use 1.5 unit of amboceptor as titrated in the presence of 0.2 c.c. of 1:10 dilution of complement serum. Titration of the hemolytic amboceptor is done as follows: into a suitable test tube rack put eight suitable test tubes, designated as Tubes Nos. 1, 2, 3, 4, 5, 6, 7 and 8, as shown in Table III. Into each tube put 0.2 c.c. of physiologic salt solution. Dilute the amboceptor serum 1:6.25 with physiologic salt solution, and add 0.2 c.c. of the diluted amboceptor to Tube No. 1. Mix the contents of Tube No. 1 and transfer 0.2 c.c. from Tube No. 1 to Tube No. 2 and so continue making dilutions until each tube contains 0.2 c.c. of diluted amboceptor. To each tube add 0.2 c.c. of 1:40 suspension of washed blood corpuscles. Put the tubes into the incubator at 37.5° C. for thirty minutes. While the corpuscles are being sensitized, shake the tubes at intervals of ten minutes and mix the complement and 50 per cent glycerol as follows: into a small flask or a large test tube put 2.5 c.c. of complement serum diluted 1:10, 2.5 c.c. of 50 per cent solution of glycerol and 5 c.c. of physiologic salt solution. After the red corpuscles have been sensitized for thirty minutes, add 0.8 c.c. of the complement-glycerol mixture, shake the tubes well, and put them into a water-bath at 37.5° C. for thirty minutes, shaking them at intervals of ten minutes. After thirty minutes in the warm bath let them stand at room temperature for thirty minutes without shaking and read the results. The last tube which shows complete hemolysis contains one hemolytic

TABLE III

TITRATION OF HEMOLYTIC AMBOCEPTOR

NO. OF TUBE	1	2	3	4	5	6	7	8
Amboceptor, 0.2 c.c. diluted	1:12.5	1:25	1:50	1:100	1:200	1:400	1:800	1:1600
Corpuscle suspension, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
In the incubator at 37.5° C. for thirty minutes.								
Complement serum, 1:10, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Physiologic salt solution, c.c.	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Glycerol 50 per cent, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
In the water-bath at 37.5° C. for thirty minutes and at room temperature for thirty minutes. Read results.								
Results	0	0	0	0	0	0	±	+

unit of amboceptor. Table III shows complete hemolysis at a dilution of 1:400 and only slight hemolysis at a dilution of 1:800. Tube No. 6 (1:400) contains about one hemolytic unit of amboceptor.

READING RESULTS

Results are read and reported in terms of fixing units per cubic centimeter of human serum tested. In previous reports, I[16, 17] described the fixing unit as the smallest quantity that will completely fix the complement contained in 0.1 c.c. of guinea pig serum, using portions of 1 c.c. of each ingredient in the test. If 1 c.c. of a 1:108 solution of the human serum contains the smallest quantity of serum which completely fixes the complement contained in 1 c.c. of a 1:10 dilution of guinea pig serum, the human serum is said to contain 108 fixing units per c.c. In daily work, I use 0.2 c.c. quantities of all ingredients and the highest dilution of the human serum, which completely fixes the test dose of complement serum, corresponds to the number of fixing units per c.c. as is shown in Tables IV and IV-A.

SENSITIVENESS

I have compared this method with the Kolmer modification of the Wassermann test, with the Kahn precipitation test and with the Meinicke precipi-

TABLE IV

READING RESULTS BY THE ROUTINE METHOD

NO. OF SERUM		CONTROL TUBE	ANTIGEN TUBES					RESULTS
	NO. OF TUBE	1	2	3	4	5	6	
	Serum dilution	1:4	1:4	1:12	1:36	1:108	1:324	UNITS PER C.C.
1	Readings	0	+	+	0	0	0	12
2	Readings	0	+	+	+	0	0	36
3	Readings	0	+	+	+	+	0	108

TABLE IV-A

READING RESULTS BY THE PRECISION METHOD

NUMBER OF SERUM	DILUTION OF SERUM	READINGS						UNITS PER C.C.
		ANTIGEN TUBES			CONTROL TUBES			
		1	2	3	1′	2′	3′	
1	1:4	0	±	+	0	0	±	4
2	1:4	±	+	+	0	0	±	8
3	1:50	0	±	+	0	0	±	50
4	1:50	±	+	+	0	0	±	100

tation test and in previous reports,[18, 19, 20, 21] I showed that I find my method more sensitive than the other methods mentioned.

METHODS

I have the routine method and the precision method. Each can be used with blood serum or with cerebrospinal fluid. Larger quantities of cerebrospinal fluid are used than of blood serum.

Procedure

Prepare three different glycerol solutions as follows:

Glycerol No. 1.—To 500 c.c. of chemically pure glycerol, add 4.5 gm. of chemically pure sodium chloride and let it dissolve.

Glycerol No. 2.—To 100 c.c. of glycerol No. 1, add 50 c.c. of physiologic salt solution.

Glycerol No. 3.—To 100 c.c. of glycerol No. 1, add 100 c.c. of physiologic salt solution.

Sterilize these three solutions.

Human Serum.—Put 0.2 c.c. of clear serum into a test tube and place it into a water-bath at about 56° C. for thirty minutes. Remove the tube with the serum from the water-bath, let it cool for a few minutes and add 0.6 c.c. of glycerol solution No. 2. This dilutes the human serum 1:4 and the glycerol content is 50 per cent. Note that the serum is heated before the glycerol is added. I find that when raw serum is mixed with glycerol, and then heated, the mixture frequently becomes anticomplementary in a few days, while this does not happen as a rule when the serum alone is first heated. Any further diluting is done with glycerol Solution No. 3, always keeping the percentage of glycerol and of sodium chloride uniform.

Antigen.—Into a 25 c.c. graduated cylinder, put 0.1 c.c. of so-called antigen (alcoholic extract of syphilitic human heart muscle or of normal beef heart muscle to every 100 c.c. of which 25 mg. of cholesterol have been added). Add 0.1 c.c. of physiologic salt solution and shake, add another 0.1 c.c. of physiologic salt solution and shake. Repeat this until 20 times 0.1 c.c. of physiologic salt solution has been added. Repeat twenty times with 0.2 c.c. portions of physiologic salt solution after which add 0.4 c.c. portions of physiologic salt solution until the total quantity measures 8 c.c. Briefly, it is done as follows: 20×0.1 c.c. of physiologic salt solution plus 20×0.2 c.c. of physiologic salt solution plus 5×0.4 c.c. of physiologic salt solution equals about 8 c.c.

Complement Serum.—The complement serums have been selected, they have been pooled and 360 mg. of chemically pure sodium chloride have been added for every 10 c.c. of serum. The salted complement serum is kept in the ice cold water-bath.

Add 1 c.c. of salted complement serum to 4 c.c. of sterile distilled water and add 5 c.c. of physiologic salt solution. This dilutes the complement serum 1:10 and the sodium chloride concentration is 0.9 per cent.

At this stage, set up the test, after that wash the corpuscles, dilute the hemolytic amboceptor, titrate and adjust the hemolytic system.

Blood Corpuscles.—Wash human blood corpuscles three or four times with physiologic salt solution, allowing the centrifuge to run ten minutes the last time to pack the corpuscles well. Read the volume of packed corpuscles and make a 1:40 (2.5 per cent) suspension in physiologic salt solution.

Hemolytic Amboceptor.—The hemolytic amboceptor is the blood serum of a rabbit which was immunized to human blood corpuscles. After bleeding the rabbit, the serum was centrifuged until clear, it was heated to 56° C. for thirty minutes and was mixed with an equal volume of glycerol No. 1. At the preliminary titration, it was found that 0.2 c.c. of 1:400 dilution contained about 1 hemolytic unit.

Add 0.1 c.c. of amboceptor serum to 35 c.c. of physiologic salt solution and mix well. Take three test tubes, into the first tube put 0.2 c.c. of diluted

amboceptor, into the second tube put 0.133 c.c. and into the third tube put 0.1 c.c. of diluted amboceptor. With physiologic salt solution, bring all volumes up to 0.2 c.c. per tube. To each tube add 0.2 c.c. of corpuscle suspension, and put the tubes into the incubator at 37.5° C. for thirty minutes, shaking them at intervals of about ten minutes.

Into a suitable test tube, put 0.8 c.c. of diluted complement serum, 1.6 c.c. of physiologic salt solution and 0.8 c.c. of glycerol solution No. 3. Mix well and add 0.8 c.c. to each tube of sensitized corpuscles. Put the tubes into the warm water-bath at 37.5° C. for thirty minutes, shaking them at intervals of ten minutes. Remove the tubes from the water-bath, let them stand quietly at room temperature for thirty minutes and read the results. If the corpuscles in the first and second tubes are dissolved with only slight hemolysis in the third tube, the dilution is correct, every 0.2 c.c. contains 1.5 hemolytic unit and that is the test dose. Before adding to the test proper, mix equal parts of corpuscle suspension and diluted hemolytic amboceptor, and sensitize for thirty minutes.

THE TEST (ROUTINE METHOD)

For each human serum to be tested, put six suitable test tubes into a suitable test tube rack, and designate the tubes as Nos. 1, 2, 3, 4, 5, and 6. Let Tube No. 1 be the control tube and Tubes Nos. 2, 3, 4, 5, and 6 the antigen tubes. Into each of Tubes Nos. 3, 4, 5, and 6 put 0.2 c.c. of 50 per cent solution of glycerol (Solution No. 3). Into Tubes Nos. 1 and 2, put 0.2 c.c. of human serum diluted 1:4 and into Tube No. 3, put 0.1 c.c. of human serum diluted 1:4. Mix the contents of Tube No. 3, and transfer 0.1 c.c. of the contents to Tube No. 4; mix the contents of Tube No. 4 and transfer 0.1 c.c. to Tube No. 5; mix and transfer 0.1 c.c. to Tube No. 6; mix and discard 0.1 c.c.

The first dilution being 1:4 and the other dilutions being multiplied by 3, the dilutions in the different tubes are as follows: Control tube 1:4, antigen tubes 1:4, 1:12, 1:36, 1:108, 1:324. These are the dilutions I mostly use for serums, but other dilutions may be used without changing the results.

Add 0.2 c.c. of physiologic salt solution to each control tube, and 0.2 c.c. of diluted antigen to each antigen tube and shake them. Add 0.2 c.c. of complement serum diluted 1:10 and 0.2 c.c. of physiologic salt solution to each tube (the complement serum may be diluted 1:20 and 0.4 c.c. may be added to each tube when human serums only are to be tested, but in testing cerebrospinal fluid, these must be added separately as will be seen later). Shake the tubes well and put them into ice cold water for at least five hours, shaking them well about two hours after putting them into the cold water.

Wash blood corpuscles and prepare a 1:40 (2.5 per cent) suspension in physiologic salt solution.

Titrate the hemolytic amboceptor in the presence of 0.2 c.c. of complement serum diluted 1:10, 0.2 c.c. of Glycerol Solution No. 3 and 0.2 c.c. of the 2.5 per cent suspension of washed corpuscles as described above. The smallest quantity of amboceptor which dissolves the corpuscles in one hour is called one hemolytic unit. Dilute the hemolytic amboceptor so that 0.2 c.c. contains 1.5 hemolytic unit.

At about thirty minutes before the hemolytic amboceptor and the blood corpuscles are to be added to the test, mix equal parts of diluted hemolytic amboceptor and corpuscle suspension, shake well, put the mixture into the incubator at about 37.5° C. for thirty minutes, shaking at intervals of about ten minutes.

Transfer the racks with the tubes containing the serum-antigen-complement mixture from the cold water-bath to the warm water-bath at about 37.5° C., after about five minutes warming, add 0.4 c.c. of amboceptor-corpuscle mixture (sensitized corpuscles) to each tube, shake them well, and return the racks with the tubes to the warm water-bath for thirty minutes, shaking them at intervals of ten minutes. Remove the racks with the tubes from the warm water-bath, let them stand quietly at room temperature for thirty minutes and read the results. The "set-up" for serum by the routine method is shown in Table V.

When testing cerebrospinal fluid, I begin with undiluted, heated cerebrospinal fluid. With cerebrospinal fluid, the dilutions in the different tubes are 0, 0, 1:3, 1:9, 1:27, 1:81. Heat 0.6 c.c. of cerebrospinal fluid. Put 0.1 c.c. of heated fluid into a test tube, add 0.1 c.c. of Glycerol Solution No. 3 and 0.1 c.c. of Glycerol No. 1. This dilutes it 1:3.

Put six suitable test tubes into a suitable test tube rack, and call them Tubes Nos. 1, 2, 3, 4, 5, and 6. Into each of Tubes Nos. 1, 2, 4, 5, and 6, put 0.2 c.c. of Glycerol Solution No. 3. Put 0.2 c.c. of undiluted fluid into each of Tubes Nos. 1 and 2. Put 0.2 c.c. of cerebrospinal fluid diluted 1:3 into Tube No. 3 and 0.1 c.c. into Tube No. 4. Mix the contents of Tube No. 4 and transfer 0.1 c.c. to Tube No. 5; mix and transfer 0.1 c.c. to Tube No. 6; mix and discard 0.1 c.c. Complete the "set-up" in accordance with Table VI.

TABLE V

"SET-UP" FOR SERUM BY THE ROUTINE METHOD

NO. OF TUBE	1	2	3	4	5	6
0.2 c.c. of human serum diluted	1:4	1:4	1:12	1:36	1:108	1:324
Physiologic salt solution, c.c.	0.2	0	0	0	0	0
Diluted antigen, c.c.	0	0.2	0.2	0.2	0.2	0.2
Complement serum diluted 1:20, c.c.	0.4	0.4	0.4	0.4	0.4	0.4
In the ice cold water-bath for five hours and in the warm water-bath five minutes.						
Sensitized blood corpuscles, c.c.	0.4	0.4	0.4	0.4	0.4	0.4
In the water-bath at 37.5° C. for thirty minutes and at room temperature for thirty minutes. Read results.						

TABLE VI

"SET-UP" FOR CEREBROSPINAL FLUID BY THE ROUTINE METHOD

NO. OF TUBE	1	2	3	4	5	6
0.2 c.c. cerebrospinal fluid, diluted	0	0	1:3	1:9	1:27	1:81
Physiologic salt solution, c.c.	0.2	0	0.2	0.2	0.2	0.2
Diluted antigen, c.c.	0	0.2	0.2	0.2	0.2	0.2
Complement serum diluted 1:10, c.c.	0.2	0.2	0.2	0.2	0.2	0.2
In the ice cold water-bath for five hours and in the warm water-bath five minutes.						
Sensitized blood corpuscles, c.c.	0.4	0.4	0.4	0.4	0.4	0.4
In the water-bath at 37.5° C. for thirty minutes and at room temperature for thirty minutes. Read results.						

THE PRECISION METHOD

Prepare the human serum, the antigen and the hemolytic system as for the Routine Method. Prepare three different dilutions of complement serum, for serum dilute the complement serum 1:10, 1:20, 1:40 and use 0.4 c.c. as the test dose. For cerebrospinal fluid, dilute the complement serum 1:5, 1:10, 1:20 and use 0.2 c.c. as the test dose.

THE PRECISION METHOD FOR SERUM

Put six suitable test tubes into a suitable test tube rack and designate them as Tubes Nos. 1, 2, 3, 1′, 2′ and 3′. Into each tube, put 0.2 c.c. of human serum diluted 1:4. Add 0.2 c.c. of diluted antigen to each of Tubes Nos. 1, 2 and 3 and 0.2 c.c. of physiologic salt solution to each of Tubes Nos. 1′, 2′ and 3′. To each of Tubes Nos. 1 and 1′, add 0.4 c.c. of complement serum diluted 1:10, to each of Tubes Nos. 2 and 2′, add 0.4 c.c. of complement serum diluted 1:20 and to each of Tubes Nos. 3 and 3′, add 0.4 c.c. of complement serum diluted 1:40. Shake the tubes well and finish the test as by the Routine Method. The "set-up" is shown in Table VII.

CEREBROSPINAL FLUID BY THE PRECISION METHOD

Heat 1.5 c.c. of cerebrospinal fluid to 56° C. for thirty minutes and set up the test as shown in Table VIII.

NEGATIVE CONTROL SERUM

Save the human serums which give negative results, heat them to about 56° C. for thirty minutes and add an equal volume of Glycerol No. 1. Pool the serums.

TABLE VII

"SET-UP" FOR SERUM BY THE PRECISION METHOD

NO. OF TUBE	ANTIGEN TUBES			CONTROL TUBES		
	1	2	3	1′	2′	3′
Human serum diluted 1:4, c.c.	0.2	0.2	0.2	0.2	0.2	0.2
Physiologic salt solution, c.c.				0.2	0.2	0.2
Diluted antigen, c.c.	0.2	0.2	0.2			
Complement serum diluted 1:10, c.c.	0.4			0.4		
Complement serum diluted 1:20, c.c.		0.4			0.4	
Complement serum diluted 1:40, c.c.			0.4			0.4
Finish the test as by the routine method.						

TABLE VIII

"SET-UP" FOR CEREBROSPINAL FLUID BY THE PRECISION METHOD

NO. OF TUBE	ANTIGEN TUBES			CONTROL TUBES		
	1	2	3	1′	2′	3′
Cerebrospinal fluid, c.c.	0.2	0.2	0.2	0.2	0.2	0.2
Glycerol 50 per cent, c.c.	0.2	0.2	0.2	0.2	0.2	0.2
Physiologic salt solution, c.c.				0.2	0.2	0.2
Diluted antigen, c.c.	0.2	0.2	0.2			
Complement serum diluted 1:5, c.c.	0.2			0.2		
Complement serum diluted 1:10, c.c.		0.2		0.2	0.2	
Complement serum diluted 1:20, c.c.						0.2
Finish the test as by the routine method.						

POSITIVE CONTROL SERUM

Save the human serums which give very strongly positive results, heat them to about 56° C. for thirty minutes and add an equal volume of Glycerol No. 1 to each. Leave them in the refrigerator for at least a month and test them again, reheating the portion to be tested. Some serums become anticomplementary to such an extent that they are unfit; these must be discarded. Pool the suitable serums, place the pooled serum in ice cold water or at lower temperature for a week and titrate it. When using it as control serum, I dilute it with Glycerol Solution No. 3 so that the test dose (0.2 c.c.) contains about three fixing units. I always reheat that portion of negative and of positive control serum to be used in connection with the Wassermann test.

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A COMPARISON OF FOUR BLOOD-SUGAR METHODS

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IN A COMPARATIVELY short period of time quantitative estimation of sugar in the blood has attained a position of definite importance in the field of clinical diagnosis and medical research. The value of these estimations lies not only in the differential diagnosis of glycosuria but the determinations also serve as a guide in scientific treatment.

As it is not always possible or practical to make a venipuncture, the present investigation was undertaken to test the relative reliability in the routine laboratory of two micromethods, using only 0.1 c.c. of blood for the determination of blood sugar, as compared with the methods at present used in the Virginia Mason Clinical Laboratory; i.e., the Myers-Bailey[1] modification of the Lewis-Benedict method and the Folin-Wu[2] method. Modifications of the latter method, which give slightly lower results, have been reported by both Benedict[3] and Folin[4] in recent years; however, the above methods have been found to give consistent results, corresponding with clinical findings.

For the blood-sugar determination in cases where two c.c. or more of blood could not be readily obtained by venipuncture (as in infants, small children, individuals with small or invisible veins, and in patients where frequent determinations may be necessary, as in diabetic coma) a simple micromethod, using only 0.1 c.c. of blood which can easily be obtained from ear or finger puncture, would be a distinct advantage. The two methods tried were a micro-modification of the Folin-Wu method after Gibson[5] and Folin's new micromethod.[6]

The procedure used in each of the four methods followed very closely the method described by the respective author.

EXPERIMENTAL

The comparison was made on 10 normal individuals and 24 patients with glycosuria. The macro estimations were made on blood from venipuncture and the micro-estimations were made on blood from ear puncture, both specimens being taken simultaneously by different technicians. Folin's new 0.1 c.c. pipettes, as sold by Eimer and Amend, N. Y., were used for both micromethods. Standardized 5 c.c. or 1 c.c. serologic pipettes were used in the macromethods. The solutions for all the methods were made up according to directions laid down by the authors in their original publications.

Fasting blood sugars were run by all four methods on 10 individuals who were normal as far as their blood sugar was concerned. Estimations were made using the new Folin micromethod both upon blood from ear puncture and upon a 0.1 c.c. portion of the venous blood used for the Myers-Bailey and

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Folin-Wu methods. The results in these cases checked very closely, with the exception of the Gibson micro-modification of the Folin-Wu method. Folin's new micromethod checked with the two macromethods when the determination was made upon the same sample of venous blood; on blood from ear puncture the values were slightly lower. The results are given in Table I.

TABLE I

COMPARISON OF BLOOD-SUGAR DETERMINATIONS ON NORMAL INDIVIDUALS

(Milligrams Per 100 c.c. of Blood)

CASE NO.	MYERS-BAILEY	FOLIN-WU	FOLIN NEW MICRO VENOUS	FOLIN NEW MICRO	GIBSON MICRO FOLIN-WU
1	154	155	154	150	161
2	110	97	102	92	88
3	112	115	112	108	100
4	97	95	94	90	85
5	125	128	128	124	103
6	102	100	103	97	94
7	117	110	114	110	100
8	110	97	110	110	94
9	98	100	101	96	88
10	123	117	125	125	105

In a sugar tolerance curve performed on a patient with exophthalmic goiter, Table II, the Myers-Bailey method gave a slightly higher curve than did any of the other methods. The values for the fasting blood sugar by the first methods gave practically the same results.

TABLE II

COMPARISON OF BLOOD-SUGAR DETERMINATIONS IN SUGAR TOLERANCE CURVE

(Milligrams Per 100 c.c. of Blood)

TIME	MYERS-BAILEY	FOLIN-WU	FOLIN NEW MICRO	GIBSON MICRO FOLIN-WU
Fasting	83	91	85	75
½ hour	250	230	244	210
1 hour	250	249	243	210
2 hours	210	188	200	175
3 hours	153	126	120	110

Table III gives the results on a series of 24 patients under treatment for diabetes and showing glycosuria. In some cases the blood was taken after fifteen hours fasting; in others, the specimen was taken one hour after lunch. Blood-sugar estimations were made by the four methods being studied.

In the first five cases single tubes with four c.c. of filtrate were used for Folin's new micromethod and the results were low; in Cases 4 and 5 the method was rerun on 2 c.c. of filtrate and checks were obtained with the other methods. In subsequent cases of the series three tubes were set up in each case instead of one. Into the first tube was pipetted 4 c.c. of the clear filtrate; into the second tube 2 c.c. of filtrate and 2 c.c. of distilled water, and into the third tube 1 c.c. of filtrate and 3 c.c. of distilled water. The three tubes were run at the same time. Accurate readings of over 200 milligrams are practically impossible with the first tube and the same is true of over 400 milligrams with the second tube. There is plenty of filtrate for this procedure, and it not

TABLE III

COMPARISON OF BLOOD-SUGAR DETERMINATIONS ON DIABETIC CASES

(Milligrams Per 100 c.c. of Blood)

CASE NO.	MYERS-BAILEY	FOLIN-WU	GIBSON MICRO FOLIN-WU	FOLIN NEW MICRO	REMARKS
1	484	475	510	206	Fasting
2	460	450	467	225	Fasting
3	420	468		244	Fasting
4	374	374	426	250	Fasting
4a				367	Rerun with 2 c.c. filtrate
5	367	380	378	248	Fasting
5a				355	Rerun with 2 c.c. filtrate
6	300	312	350	278	One hour after lunch
7	300	307	290	300	Fasting
8	187	185	214	173	Fasting
9	180	162	181	190	One hour after lunch
10	235	214	272	200	Fasting
11	211	185	200	222	One hour after lunch
12	300	280	250	285	Fasting
13	187	185	166	181	Fasting
14	166	170	162	166	Fasting
15	154	155	161	150	Fasting
16	284	296	270	278	Fasting
17	120	115	112	125	Fasting
18	600	590		664	Coma
19	600			666	Coma
20	194			170	Fasting
21	88	85		80	Fasting
22	200	193		177	Fasting
23	166	152		160	One hour after lunch
24	230	228		212	Fasting

Only one tube containing 4 c.c. of filtrate was used in the first determinations by the new Folin micromethod. In all subsequent determinations three tubes were set up simultaneously.

only saves a great deal of time but also removes the doubt from determinations which are not being checked by some other method and are near the limit of accuracy of the new Folin method.

The Folin-Wu method and the Myers-Bailey modification of the Lewis-Benedict method checked very closely, many of the slight variations being less than experimental error of the methods. The new Folin micromethod paralleled the two macromethods, giving values averaging a few milligrams lower than the other two methods. However, the differences were not sufficiently great to be significant in clinical interpretation. In Cases 18 and 19 difficulty was experienced in securing sufficient blood by venipuncture for the macromethods, so that the higher values of the micromethod are probably the more accurate. The Gibson micro-modification of the Folin-Wu method showed greater and less consistent variations and consequently was discarded.

SUMMARY

1. Folin's new micromethod for blood-sugar determination gave results in excellent agreement with the older macromethods on both normal and diabetic blood.

2. Folin's new micromethod checked very closely with the Folin-Wu method and Myers-Bailey modification of the Lewis-Benedict method when the determination was made on the same sample of venous blood. On blood

from ear puncture the values were a few milligrams lower than on venous blood drawn at the same time.

3. Setting up three tubes with varying amounts of filtrate in place of one in Folin's new micromethod was found not only to save a great deal of time in the clinical laboratory, but gives assurance to the determinations, removing the doubt from those determinations showing values near the limit of accuracy of the method.

4. The Gibson micro-modification of the Folin-Wu method gave results which were considered to have too great and too inconsistent a variation from the average of the other three methods.

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A NEW TYPE OF TISSUE CRUSHER*

By Allen C. Nickel, M.D., Rochester, Minn.

THE usual method of culturing surgically removed tissue, or tissue removed in a sterile manner, by grinding in mortars, is tedious and offers sources for contamination. I have employed this method for years, using sand as the abrasive. This is fairly reliable as long as the material to be ground is relatively soft and easily disintegrated, such as liver tissue, but when dense fibrous tissue, such as a uterine fibroid tumor, is to be ground, it is almost impossible to macerate it in a mortar without its becoming contaminated.

Rosenow,† in 1914, reported on the use of two methods, or appliances, which effectively eliminated the usual sources of contamination. One of these methods consisted of grinding the tissue in a sterile air chamber which contained a small meat chopper and a mortar. The tissue was ground either in the mortar alone or, if it was particularly tough or large in size, it was first passed through the meat chopper and then ground in the mortar. The hand was placed in a sterilized glove, the outer surface of which formed part of the lining of the sterile chamber. However, this method was rather cumbersome and bulky when numerous cultures were required. At the same time, Rosenow reported on an all-metal device by which the tissue that was to be emulsified was forced by means of a threaded hollow plunger through numerous small holes of a disk. This method, although it eliminates sources of contamination,

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†Rosenow, E. C.: The Newer Bacteriology of Various Infections as Determined by Special Methods. Jour. Am. Med. Assn., 1914, lxiii, 903-907.

also has its practical drawbacks; the small holes are hard to clean and there is a tendency for the tissue to be forced backward along the screw threads.

Several years ago, Rosenow used another method for handling extracted teeth. He caused to be constructed a small hollow metal cylinder and a metal plunger which fitted it. He placed the extracted tooth in the cylinder, and by pounding on the plunger the tooth was crushed. From the débris, the pulp or apex could be picked out for culture.

Using this instrument, I attempted to crush tissue but was unable to accomplish anything because of the resiliency of the tissue. I then thought of using a grinder resembling a pestle working in a mortar but employing an eccentric axis. The method was similar to that used in grinding eye-glasses. However, the tissue would work to the side, away from the grinding area.

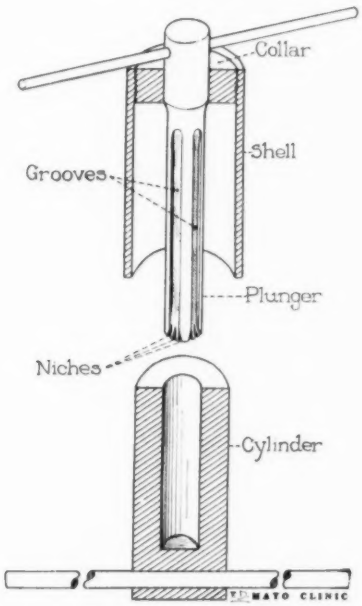

Fig. 1.—Tissue crusher.

Then the idea occurred to me that if the outlets for the crushed tissue were made on the periphery of the plunger instead of in the cylinder head, there would be only one avenue of escape for the tissue. This, with several modifications to overcome technical difficulties that arose, led to the development of the present instrument (Fig. 1). An instrument of the size described in this article has been very satisfactory for the majority of the tissues from which cultures have been made.

The instrument consists essentially of two parts: the first part is a hollow cylinder; the second part is a combined plunger and shell which fit respectively into the cylinder and over the outside of it. The cylinder is made of a bar of monel metal one inch in diameter and two and a fourth inches long in which is drilled a hole three-eighths of an inch in diameter and one and three-fourths inches deep. This leaves the base thick enough for a three-sixteenth inch hole to be drilled crosswise through it. A steel rod is pushed through

this hole for leverage. The plunger consists of a solid, steel, nickel-plated cylinder three and a fourth inches long and three-eighths of an inch in diameter; it fits snugly, yet slides easily into the hollow cylinder. The steel shell that has been mentioned is two and an eighth inches long, $1\frac{5}{32}$ inches wide over all and approximately one-thirty-second of an inch thick; it screws onto a collar three-eighths of an inch thick, which is fixed on the plunger so as to leave two and three-eighths inches of the plunger below the lower surface of the collar. On the plunger are four grooves which run lengthwise and which are equidistant from each other; each groove is one-eighth of an inch wide, about one-twenty-fifth of an inch deep, two and a fourth inches long, and begins one-eighth of an inch from the lower end of the plunger. On this lower one-eighth of an inch of the plunger are twelve small nitches made with a three-cornered file; they are about one-thirty-second of an inch wide and one-thirty-second of an inch deep. As seen in cross-section, on the lower face of the plunger, they are approximately equidistant from each other, but they are so placed that each three nitches converge and lead into each of the four long vertical grooves just described. At the opposite end of the plunger is a crosswise hole an eighth of an inch in diameter similar to the hole in the base of the cylinder; through this hole a rod is inserted for leverage.

After the instrument has been sterilized in the autoclave, it is used as follows: A piece of tissue to be cultured is inserted in the cylinder, with sterile forceps or scissors, and is pushed toward the bottom; air pockets are avoided if possible. The plunger is then inserted as far as it can be shoved easily. The sterile towel in which the instrument was wrapped is wrapped especially around the lower edge of the shell and the entire instrument is then placed in a vise. Pressure is made on the top of the plunger and base of the cylinder, and as the vise is screwed shut the tissue is forced through the twelve fine grooves, up along the four parallel larger grooves and into the base of the shell. The vise is then loosened, the two metal rods are inserted into the holes in the cylinder and plunger and then rotated, if necessary, to loosen the plunger from the cylinder. The sterile towel is then removed, the plunger, with its shell, is removed from the cylinder after the lower edge of the shell has been flamed, and the macerated tissue is found along the grooves and in the bottom of the cup formed by the shell and the collar on the plunger. With a sterile pipette and salt solution, the macerated tissue is washed from the plunger and diluted in the cup and from there it is pipetted into the desired culture mediums. It is advisable to tilt the vise backward slightly from the usual horizontal plane so that the tissue juices will gravitate to the base of the shell.

The plunger, from the lower end to the lower surface of the collar, is made five-eighths of an inch longer than the hole in the cylinder, so that there is space provided for the macerated tissue when the plunger is driven home. The shell is made large enough to provide a clearance of about three-sixty-fourths of an inch between it and the cylinder. When the plunger is driven home, the lower edge of the shell is one and one-eighth inches from the base of the cylinder. This provides ample space to take hold of the cylinder without contaminating the lower edge of the shell. It is desirable to mark on the

cylinder the position of the lower edge of the shell when the plunger is in the cylinder as far as possible so that undue pressure shall not be made after the plunger is at the bottom of the shaft. Otherwise, it is likely to be bent. If a very sinewy piece of tissue seems to resist crushing it sometimes can be crushed by closing the vise in jerks and waiting a moment between each partial turn of the vise handle. Originally, it was thought advisable to hollow the upper end of the plunger and insert a ball bearing to provide rotation of the plunger while closing the vise, but so far this has not been needed. The twelve small nitches in the plunger can be filed to suit the tissue to be cultured. However, I have found that nitches which are very small give very fine maceration but require tremendous power to close the vise. Under these conditions, the plunger often is bent; this makes much more work and sometimes the tissue is contaminated in attempts to remove the bent plunger. For this reason, also the plunger is made of steel, since it is harder than monel metal. The nickel-plating is used to prevent rusting.

Crushing the tissue evidently does not kill the organisms since resected ulcers and diseased gall bladders cultured in this manner may yield just as good a culture of pathogenic organisms as that obtained with the mortar and pestle. Moreover, in cultures from tissue macerated with the mortar and pestle the incidence of organisms indicating contamination was higher than that when the tissue crusher just described was used. I hope that further investigation with known cultures will confirm the fact that the tissue crusher lessens the chances for contamination.

A SIMPLE AND ACCURATE HEMATOCRIT*

By M. M. Wintrobe, M.D., New Orleans, La.

THE determination of cell volume, the volume of packed red cells in a given sample of blood, is a most valuable procedure in the diagnosis and differentiation of the anemias. From the red cell count, hemoglobin and cell volume, the volume index and the saturation index can be determined[1, 2] and the volume of the average red corpuscle, as well as its hemoglobin content, calculated.[3] The determination of cell volume has not been generally adopted as a laboratory diagnostic procedure principally because of lack of appreciation of the information to be derived and, secondly, because a simple and yet accurate hematocrit has not been available. A hematocrit which meets these requirements is here described.

The instrument (Fig. 1, *A*) is a narrow glass tube 11 cm. in length, of even bore (3 mm. inside bore), and with a flat bottom. A centimeter-millimeter scale, commencing at the level of the inside bottom of the hematocrit, is etched on the glass.†

*From the Department of Medicine, Tulane University School of Medicine, New Orleans. Received for publication, July 1, 1929.

†The instruments have been made for us by Corning Glass Works, Corning, N. Y.

Blood for the hematocrit is drawn from an arm vein in the usual manner and collected in a test tube. Ten milligrams of dry neutral potassium oxalate in the bottom of the tube is sufficient to prevent the coagulation of 5 c.c. of blood. Oxalate added in this proportion causes a shrinkage in cell volume of 3.7 per cent and an appropriate correction for this shrinkage should be made.[2] Instead of solid oxalate, a 20 per cent solution may be used as the anticoagu-

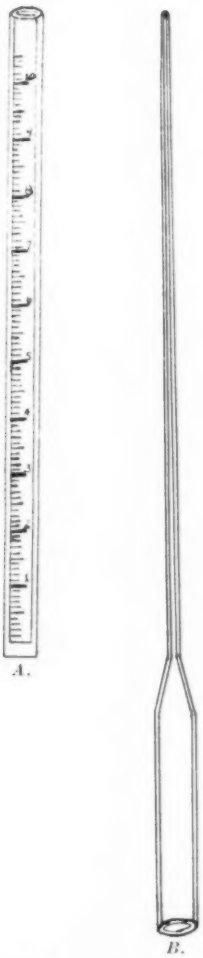

Fig. 1.—*A*, Hematocrit. *B*, Pipette for filling hematocrit.

lant. One drop of such a solution, measured by a pipette which delivers 20 drops to the cubic centimeter, will prevent the coagulation of 5 c.c. of blood and causes a shrinkage in cell volume of approximately 3 per cent.

A little less than 1 c.c. of blood is required for the hematocrit determination. The blood is transferred to the hematocrit from the test tube by means of a pipette made from glass tubing drawn out to such a length that the pipette can be passed to the bottom of the hematocrit (Fig. 1, *B*). These pipettes are easily made at a nominal cost (½ to 1 cent each).

In order to prevent evaporation of serum during centrifugation, the hematocrit should be stoppered by means of a rubber cork. No special centrifuge attachment is required for the hematocrit, the ordinary centrifuge tube holder being quite satisfactory. It is important to centrifugalize long enough to secure complete packing. Centrifugation at 3000 revolutions per minute for fifteen minutes is usually sufficient, but when great accuracy is desired, this should be carried on for thirty minutes. The time required for complete packing is easily determined for any centrifuge by repeated centrifugation for different lengths of time until no further decrease in volume of packed cells is noted.

The cell volume in per cent is determined by dividing the height of the column of packed red cells by the total height of the column of cells and serum, and multiplying by 100.

The hematocrit is easily cleaned by means of pipettes similar to those employed for filling it. A very convenient arrangement consists of bottles of water, alcohol and ether, each connected by a siphon system to one of these pipettes. In this way the cleansing fluid is readily forced down to the bottom of the hematocrit.

Parallel cell volume determinations using a specially calibrated centrifuge tube and this hematocrit have confirmed the accuracy of the instrument here described. This hematocrit has the advantage over the ordinary centrifuge tube in that a small quantity of blood only need be used, while the reading of cell volume is more easily and more accurately made on account of the narrow column of cells. As further proof of the accuracy of the instrument may be mentioned the fact that parallel determinations on the same sample of blood using several hematocrits agree within 1 per cent.[4] A long experience with this instrument has convinced me of its uniform accuracy.

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A NEW RAPID PARAFFIN METHOD FOR TISSUE SECTIONS*

By Ida M. Vilkomerson, New York, N. Y.

IN VIEW of the fact that an early report on tissues often is of importance, a rapid yet efficient method for running through sections is desirable. At best, present paraffin methods consume three days. Therefore to hasten such work, a series of experiments were made during which the following procedure was found to be very satisfactory, tissues being ready for diagnosis within four hours after the fresh specimen had been received.

The tissue segments should not be more than 2 mm. in thickness. It is advisable to use throughout a wide-mouthed bottle to facilitate transfer of tissue. The specimen is first placed in the following solution:

Absolute methyl alcohol	60 c.c.
Acetone (C. P.)	100 c.c.
Iodine crystals	1 to 2 crystals
Anhydrous copper sulphate	½ inch layer

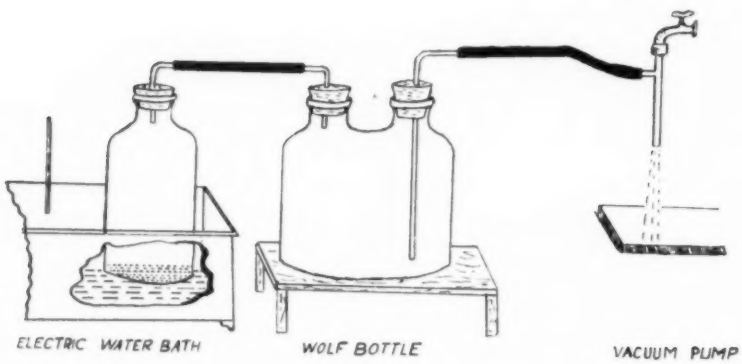

Fig. 1.

The combination of absolute methyl alcohol and acetone is ideal, as it fixes and dehydrates at the same time, whereas in all other methods this process consists of two separate steps requiring from several hours to two days. Addition of a few iodine crystals increases the penetrating power of the solution. The half-inch layer of anhydrous copper sulphate at the bottom of the bottle removes any remaining traces of water. The copper sulphate is covered with several ply of gauze, upon which the tissue is placed for one and one-half hours. It is then transferred to chloroform for thirty minutes, then to a mixture of equal parts of chloroform and paraffin for fifteen minutes.

The tissue is next placed in clean melted paraffin (55° C.) for fifteen min-

*From the Bendiner and Schlesinger Research Laboratory, New York.
Received for publication, July 31, 1929.

utes, every trace of chloroform being removed, and then transferred to a bottle containing filtered paraffin to which is attached a vacuum apparatus (see Fig. 1). The tissue remains in vacuo in the paraffin for fifteen minutes. For this purpose an electric water-bath (56° C.) may be used. This can readily be brought near a sink with a vacuum pump attachment and connected to a Wolf-bottle to prevent back-flow of water or it may be used with vacuum piped system. In using the paraffin with vacuum, there are several advantages over other paraffin methods usually requiring several hours in the oven: (1) By the vacuum method there is very much better infiltration with paraffin; (2) there is considerably less exposure to heat, thus preventing shrinkage of tissue; (3) the vacuum also helps to draw out the last traces of chloroform and therefore insures perfect imbedding; (4) much time is saved. It is important that the temperature of the water-bath should not exceed 1° C. above the melting point of the paraffin used.

After this treatment, the infiltrated tissue is handled in the usual fashion; but only ten minutes drying in an oven at 37.5° C. is necessary, if excess water is wiped off the edge of the slide. It was found unnecessary to use albumin to cause the sections to adhere to the slides, if the latter were cleansed with acid alcohol to remove grease.

SUMMARY

Running through of tissue sections:

SOLUTION 1		
Absolute methyl alcohol	60 c.c.	1½ hours
Acetone (C. P.)	100 c.c.	
Iodine crystals	1 to 2 crystals	
Anhydrous copper sulphate	½ inch layer	
SOLUTION 2		
Chloroform		½ hour
SOLUTION 3		
Chloroform-paraffin		¼ hour
SOLUTION 4		
Paraffin (plain)		¼ hour
SOLUTION 5		
Paraffin with vacuum		¼ hour
Imbed.		
		2¾ hours

CONCLUSION

By this rapid method tissues can be fixed, dehydrated, imbedded, cut, and stained in from three and a half to four hours. If attention is given to all details, it is simple and thoroughly efficient. Sections prepared by this procedure keep well and may be filed for future study.

THIRD AVENUE AT TENTH STREET.

THE VAN DEN BERGH REACTION*

A Comparison of Technics

By Harry Shay, M.D., and Eugene M. Schloss, M.D., Philadelphia, Pa.

In 1918,[1] van den Bergh introduced his method for determining the presence of bile pigment in body fluids. No one can deny the importance of this reaction in recent studies of jaundice and the stimulus it gave to renewed investigation of many liver conditions. A method for the detection of bile pigment far more delicate than any previously suggested, it has the added advantage of being specific for bilirubin. This specificity readily establishes its superiority over the icterus index, inasmuch as it is not quantitatively altered by hemolysis, lipemic opalescence,[2] or the presence of other pigments, such as lutein or carotin. The reaction differs also from all other methods of estimating the bilirubin content of body fluids, in its value in indicating the obstructive and nonobstructive types of bilirubin. The theory upon which this qualitative differentiation is based is described in papers by McNee[3] and by Bockus and Shay[4] and need not be entered into here.

We wish to present observations on some modifications of technic which, though far superior to the original methods, have, for some reason, not come into general usage. We would call especial attention to the modifications suggested by Lepehne[5] for the qualitative reaction and by Thannhauser and Andersen[6] for the quantitative. Greene, Snell and Walters[7] mention the superiority of the latter but do not, we believe, sufficiently stress it.

THE QUALITATIVE REACTION

The original qualitative technic carried out by mixing equal quantities of blood serum and reagent, makes it difficult for the operator to judge accurately the early production of color, the production of lesser degrees of color, and the end-point of the reaction. In Lepehne's modification, three small tubes are used, in each of which is placed 0.25 c.c. of blood serum. To Tube I is added 0.2 c.c. of water; to Tube III, 0.2 c.c. of diazo reagent. The tubes are set aside for at least fifteen minutes to allow for completion of the reaction in Tube III. At the end of this interval, 0.2 c.c. of diazo reagent is added to Tube II, and the color, time of appearance of the color, and time of complete reaction are noted, Tubes I and III serving as controls for the negative and completed reactions respectively.

We have graded our reactions according to the time limits suggested by McNee[3] but have also adopted the biphasic classification of Feigl and Querner.[8] These include:

1. *Prompt Direct Reaction:* Bluish violet color beginning immediately upon mixing serum and reagent and becoming maximal in thirty seconds.

*From the Gastrointestinal Clinic of the Jewish Hospital.
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2. *Delayed Direct Reaction:* Reddish coloration, beginning only after one to fifteen minutes, gradually deepening to more violet.

3. *Biphasic Direct Reaction:* Reddish color appearing at once and either slowly or rapidly deepening to violet.

4. *Negative Reaction:* No color appearing within fifteen minutes.

Utilizing this technic, we cannot agree with Andrewes[9] or with Hall[10] that the maximum color is not often reached in thirty seconds in direct immediate sera. While it is impossible to present graphic data for the qualitative reaction, we are convinced that the modified technic enables one to classify far more accurately the reactions obtained. It is particularly useful in cases of mild jaundice where the intensity of the color reaction produced is not very pronounced even when complete. Not infrequently definite direct immediate or delayed reactions can be detected where the use of the single tube technic would place them in the indirect or negative group. The superiority of this method lies in the fact that it establishes controls for the two variables in the reaction; namely, the color production and its production time.

THE QUANTITATIVE REACTION

The original quantitative technic is so frequently described in the literature that it is unnecessary to do so here. Van den Bergh from the start realized that this was not an accurate method and has always called it an "estima-

TABLE I

A COMPARISON OF BLOOD BILIRUBIN READINGS BY THE STANDARD VAN DEN BERGH TECHNIC AND BY THE THANNHAUSER AND ANDERSEN MODIFICATION

Serum

CASE NO.	DIAGNOSIS	VAN DEN BERGH ORIGINAL TECHNIC	THANNHAUSER AND ANDERSEN MODIFICATION	CONFIRMATION OF DIAGNOSIS
1	Carcinoma of tail of pancreas	0.2	0.7	Operation
2	Carcinoma of tail of pancreas	0.3	0.3	Operation
3	Cirrhosis of liver	0.3	0.5	
4	Carcinoma of stomach	0.3	1.0	Operation
5	Chronic cholecystitis	0.3	1.3	Operation
6	Catarrhal jaundice	0.3	1.5	
7	Carcinoma of cecum	0.4	0.5	Operation
8	Cholelithiasis	0.5	0.6	Operation
9	Cholelithiasis	0.5	0.7	Operation
10	Cholelithiasis	0.5	1.7	Postmortem
11	Cholelithiasis	0.5	2.3	Operation
12	Carcinoma of head of pancreas	0.6	3.4	Operation
13	Catarrhal jaundice	0.7	3.2	
14	Catarrhal jaundice	0.7	5.0	
15	Carcinoma of head of pancreas	1.7	4.1	Operation
16	Hemolytic jaundice	2.0	4.6	Operation
17	Carcinoma of head of pancreas	2.5	14.2	Operation
18	Catarrhal jaundice	3.5	12.5	
19	Cholelithiasis	5.0	15.0	Operation
20	Carcinoma of head of pancreas	5.2	17.5	Operation
21	Carcinoma of stomach and liver	6.4	19.3	X-ray
22	Catarrhal jaundice	7.7	9.3	
23	Carcinoma of head of pancreas	9.2	30.3	Operation
24	Catarrhal jaundice	10.8	32.0	
25	Catarrhal jaundice	12.8	35.2	
26	Carcinoma of head of pancreas	14.6	26.7	Operation
27	Carcinomatosis	16.6	25.4	Operation
28	Cholelithiasis	19.5	58.0	Operation

All readings in van den Bergh units.

tion." The discrepancy in the reaction is due to the fact that some bilirubin is lost in the albuminous precipitate produced in the test. While it is true that the greatest loss occurs with sera giving a strong prompt direct reaction (see Table I), we cannot agree with McNee and Keefer[11] that this loss may be disregarded for sera giving a delayed direct reaction. An appreciable difference (133 per cent) by the old and modified technics, has even been noted in the blood from a case of true hemolytic jaundice (Table I, Case 16).

The modification of Thannhauser and Andersen is superior in that all of the azo-bilirubin can be retained for estimation in the supernatant liquid. This is accomplished by first mixing the test serum and diazo reagent, allowing coupling to become completed, and then precipitating the albumin with alcohol and saturated ammonium sulphate. One can readily see the difference between the old and new technics by noting the color of the precipitate after centrifuging. In the former especially, if the serum is heavily jaundiced, the precipitate is always definitely yellow, while by the latter method, the precipitate is *white*. The explanation for this difference in reaction may lie in the far greater solubility of azo-bilirubin in alcohol as compared to that of pure bilirubin.

The details of the modified technic are as follows: To 1 c.c. of serum add 0.5 c.c. of diazo reagent. After allowing coupling to take place (we prefer fifteen minutes), add 2.5 c.c. of 95 per cent alcohol and 1 c.c. of a saturated solution of ammonium sulphate. These are mixed and centrifuged. The mixture separates into three layers, a lower clear watery one, a middle white layer of precipitate, and an upper, clear, reddish violet layer containing the azo-bilirubin. The upper layer is removed and compared colorimetrically with a standard. In our comparative studies we used van den Bergh and Muller's cobalt sulphate standard. This is made up by dissolving 2.161 gm. of *anhydrous* cobaltous sulphate in 100 c.c. of distilled water. This solution gives a color corresponding to one unit of bilirubin and is permanent if kept in the dark. Table I shows a comparison of the old and new methods in a series of 28 determinations in a variety of diseases.

TABLE II

SAME AS TABLE I EXCEPT THAT READINGS ARE TAKEN ON THE BLOOD PLASMA INSTEAD OF ON THE SERUM

Plasma

CASE NO.	DIAGNOSIS	VAN DEN BERGH ORIGINAL TECHNIC	THANNHAUSER AND ANDERSEN MODIFICATION	CONFIRMATION OF DIAGNOSIS
1	Cholelithiasis	0.3	0.5	Operation
2	Cholelithiasis	0.5	2.1	Postmortem
3	Carcinoma of head of pancreas	1.6	3.7	Operation
4	Carcinoma of head of pancreas	2.5	8.8	Operation
5	Catarrhal jaundice	3.5	9.5	
6	Carcinoma of head of pancreas	3.9	10.0	Operation
7	Catarrhal jaundice	4.5	24.7	
8	Carcinoma of stomach and liver	5.1	15.1	X-ray
9	Carcinoma of head of pancreas	9.3	30.5	Operation
10	Catarrhal jaundice	8.5	33.5	
11	Carcinoma of head of pancreas	10.5	25.3	Operation
12	Carcinomatosis	15.8	18.0	Operation

Table II shows a similar difference in readings obtained by using the plasma instead of the serum.

We cannot agree with McNee and Keefer[11] that the plasma may be utilized equally as well as the serum. In our comparative series as shown in Table III we obtained consistently lower readings with the plasma as compared with the serum, when examined simultaneously. It will be noted in this group that in one instance (Table III, Case 8), the plasma reading was

TABLE III

A COMPARISON OF THE BLOOD SERUM AND PLASMA READINGS AS OBTAINED BY BOTH METHODS. NOTE THE PRACTICALLY CONSISTENT HIGHER SERUM READINGS OBTAINED BY THE MODIFIED TECHNIC[11]

CASE NO.	DIAGNOSIS	VAN DEN BERGH ORIGINAL TECHNIC		THANNHAUSER AND ANDERSEN MODIFICATION	
		SERUM	PLASMA	SERUM	PLASMA
1	Cholelithiasis	0.5	0.5	0.7	0.5
2	Cholelithiasis	0.5	0.5	1.7	2.1
3	Carcinoma of head of pancreas	1.7	1.6	4.1	3.7
4	Carcinoma of head of pancreas	2.5	2.5	14.2	8.8
5	Catarrhal jaundice	3.5	3.5	12.5	9.5
6	Carcinoma of head of pancreas	5.2	3.9	17.5	10.0
7	Carcinoma of stomach and liver	6.4	5.1	19.3	15.1
8	Carcinoma of head of pancreas	9.2	9.3	30.3	30.5
9	Catarrhal jaundice	12.8	8.5	35.2	33.5
10	Carcinoma of head of pancreas	14.6	10.5	26.7	25.3
11	Carcinomatosis	16.6	15.8	25.4	18.0

slightly higher than the serum. In this case the plasma-diazo mixture was allowed to stand overnight before precipitating with alcohol. We are inclined to believe that the time permitted for coupling to occur may have some effect on the final reading. Investigations regarding this are now going on in this clinic and will be reported if found of any importance.

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1309 SPRUCE STREET.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SPERMATOZOA COUNT: In the Diagnosis, Prognosis, and Treatment of Sterility, Macomber, D., and Sanders, M. B. New England, J. M. 200: 981, 1929.

A blood counting chamber and a white blood cell pipette are necessary. The diluent is a solution of five per cent sodium bicarbonate to which one per cent formalin has been added. The bicarbonate dissolves small amounts of mucus and the formalin is added to stop the activity of the spermatozoa. This semen is drawn up to the 0.5 mark on the pipette and then diluted with the bicarbonate-formalin solution to the 11 mark. The pipette is then thoroughly shaken to obtain a uniform mixture. A drop is placed on the counting chamber and the spermatozoa are counted in the same manner as in making a white cell count. After the number of spermatozoa in the millimeter square is determined, the following formula is used to compute the number of spermatozoa per cubic centimeters; Number in millimeter square x 10 (depth) x 20 (dilution) equals the number per cubic millimeter x 1,000 equals the number per cubic centimeter. The two chief sources of error are: in the cases where there is an excess of mucus in the semen and in cases where it is observed in the preliminary examination under the microscope that the numbers are very low. The first may be corrected by diluting 1 c.c. of semen with 19 c.c. of the bicarbonate-formalin solution, shaking thoroughly and counting without further dilution. The second may be counted directly in the counting chamber without any dilution whatever. The average normal count is 100,000,000 per c.c. (calculated from 294 cases).

MERCURY: Detection of in Skin: Discoloration of Skin Due to Mercury, Hollander, L., and Baer, H. L. Arch. Dermat. & Syph. 20: 27, 1929.

Two samples of the skin were placed on separate watch glasses, and 15 per cent nitric acid was added to each. These watch glasses were then placed on water-baths and heated at the boiling temperature of water for two hours. At the end of this time only a few yellow pieces of solid material remained in the clear solutions.

Chlorine was passed into the solutions, for fifteen minutes. To expel excess chlorine, air was passed through the chlorinated solutions until the odor of chlorine was no longer detectable. The solutions were concentrated to 1 or 2 c.c. and were electrolyzed.

In the electrolysis, platinum wires were employed as anodes and copper wires had a gauge of 30, and the ends which were to be immersed were ground to fine points and then smoothed with emery cloth. The platinum and copper wires were held in place and isolated from each other during electrolysis by means of a piece of rubber. A storage battery was used to furnish a current and a post office box furnished the necessary resistance. Electrolysis was carried out with a current of 1.5 milliamperes and 1.5 to 2 volts.

The solutions of the two samples of skin were electrolized for thirty minute periods at the same amperage and voltage. During the electrolyses, the platinum anodes were immersed to a depth of approximately 0.5 cm., but the copper cathodes were so arranged that only the points were in the solutions. The electrodes were placed as close as possible in the solutions without having actual contact. At the end of thirty minutes the current was shut off; the copper cathodes were removed, washed with water and dried with clean pieces of silk. To the naked eye the points of copper showed a characteristic blackening. These points were next examined under the microscope.

In the microscopic examination, the cathode points, which rested on glass slides coated with asphaltum, were examined under a magnification of 100 diameters. Illumination was obtained by the use of a Silverman illuminator.

The samples examined showed characteristic deposits of silver-gray mercury gathered as small globules on the tips of the copper wires. These were in marked contrast to the copper color of wires which has been polished but not subjected to electrolysis, and wires which had been used as cathodes in thirty minute electrolysis of 1 to 2 c.c. of water acidified with one drop of 15 per cent nitric acid, which were examined simultaneously on the asphaltum-coated glass slides.

As a further proof that deposits of mercury had been obtained, these wires were heated at a distance of 2 to 3 c.c. from the point which had the globules of mercury, by means of a micro-bunsen burner. The period of heating varied from 0.5 to 1 minute. The wires were cooled, and when reexamined it was found that the bright metallic globules had disappeared. This coincided with the disappearance of mercury from copper wires which had been used in the electrolysis of weak mercuric nitrate solutions ($Hg(NO_3)_2.H_2O$) and have been subjected to a similar treatment.

SPIROCHETES: Staining of in Nervous Tissue, Kanzler, R. Ztschr. f. d. ges. Neurol. u. Psychiat. **117**: 171, 1928.

The section is immersed for thirty minutes in a solution of ammonium bromide and formaldehyde. It is then washed and put in pyridine for fifteen minutes, washed again, and immersed for ten minutes in a 0.5 per cent uranin solution. After being washed again, the sections are immersed for one hour in a 1.5 per cent silver nitrate solution at from 37 to 40° C., quickly heated in the silver solution over the flame, washed in distilled water, dipped and moved about for from two to four seconds in a solution of silver nitrate, soda and ammonia. Then, without being washed, they are placed for from three to five seconds in a 5 per cent formaldehyde solution. As soon as they have taken on a yellow or yellowish-brown color, they are placed in distilled water, which is changed several times, and consecutively in alcohol, beechwood creosote-phenol-xylene, and Canada balsam. The spirochetes are stained black, whereas the nervous tissue remains unstained.

INDOL: Simple Test for, Kovacs, N. A. Ztschr. f. Immunol. u. Exper. Therap. **55**: 311, 1928.

P-dimethylamidobenzaldehyde (Merck), 5 gm. is dissolved in pure amyl alcohol, 75 c.c., and concentrated hydrochloride acid, 25 c.c.; from 25 to 30 drops of this solution are added to broth cultures of bacteria. After a gentle shaking a violet-red color appears if indol is present in the broth.

BLOOD SUGAR: The Preservation of Blood for Estimations of, Lax, H., and Szirmat, I. Munchen. med. Wchnschr. **76**: 58, 1929.

The glycolysis and bacterial destruction of the sugar in diabetic and normal blood are prevented by the addition of 1 per cent sodium fluoride and 0.1 per cent mercuric chloride. With the addition of these reagents the sugar content of the blood remained unchanged even after thirty days' storage in the incubator.

UNDULANT FEVER: Isolation of Brucella Organism from the Stools, Amoss, H. L., and Poston, M. A. J. A. M. A. **99**: 170, 1929.

About 1 gm. of fresh feces was mixed in 50 c.c. of sterile isotonic salt solution and shaken for a few minutes to insure thorough suspension. The suspension was filtered through four layers of No. 1 hospital gauze to remove gross particles and centrifugated at half speed for three minutes to throw down other particles and larger bacteria. To the supernatant suspension, a sufficient amount of immune serum was added to make the total dilution 1:100, and, after shaking, the mixture was placed in a 37° C. water-bath for two hours. The suspension was centrifugated at half speed for five minutes and the supernatant fluid discarded. The precipitate was resuspended in isotonic salt solution, stirred, and centrifugated at the same speed again. The supernatant fluid was again discarded and

the procedure repeated twice. Finally the precipitate was spread with a bent glass rod on eosin-methylene blue plates, some of which were incubated at 37° C. aerobically and others in an anaerobic jar containing 10 per cent carbon dioxide.

Large clear colonies appeared after ninety-six hours. These were fished and the organism identified in the usual manner.

In twenty experiments the patient's own serum known to agglutinate Brucella melitensis strain 428 of the Hygienic Laboratory was used to concentrate the fecal organisms. In sixteen experiments, the polyvalent antimelitensis serum produced by Mulford was employed in a dilution of 1:100 and of 1:300 with equal success.

It is suggested that, in cases from which the organism has not been recovered from the urine or the blood but in which the patient's serum agglutinates members of the Brucella group, either the patient's serum or the corresponding polyvalent or monovalent serum be used. On account of the dilution employed, the small amount of preservative may be disregarded.

HOOKWORM: Suitability of Various Bacteria as Food for Hookworm Larvae, McCoy, O. R. Am. J. Hyg. 10: 140, 1929.

The following method was used for the isolation of the ova:

Thoroughly mix up about 25 to 50 gm. of freshly passed feces in about 500 c.c. of water. An electric mechanical stirrer proved very useful for this purpose. The mixture was then washed through a series of copper wire sieves ranging up to a mesh of 100 wires to the inch. The coarse material in the feces was caught in the sieves but the eggs passed through with the filtrate. The filtrate was then allowed to stand in a large sedimenting cone for about one hour while the eggs and other heavy particles settled to the bottom. The supernatant fluid was then poured off and the sediment transferred to a 50 c.c. centrifuge tube. This sediment was repeatedly washed by centrifuging at a speed of 1,000 revolutions per minute. When the supernatant fluid from the washings became practically clear, saturated salt solution was added to the sediment and the material again centrifuged at the same speed. This time the eggs came to the surface and could be collected by removing the surface film with the open end of a piece of large glass tubing.

ASCARIS: On the Use of a Method for the Isolation of Ascaris Eggs from the Soil, Spindler, L. A. Am. J. Hyg. 10: 157, 1929.

A pint or more of soil is collected from a suspected spot by sweeping or lightly scraping the surface of the ground over a large area. In the laboratory the sample is thoroughly crushed and mixed and a representative 5 to 10 gm. portion placed in a 50 c.c. centrifuge tube and treated for an hour with 10 c.c. of 30 per cent antiformin solution. It is quite imperative, in this process, that the mixture be given frequent thorough stirrings to allow the antiformin to act on every particle of soil. If this is not done many eggs will remain adhering to the soil particles and will not be recovered in flotation. When sufficient time has elapsed for the eggs to become freed from the soil the tubes are filled with sodium dichromate (specific gravity 1.35), thoroughly shaken and then centrifuged at 1,000 r. p. m. for one or two minutes. The eggs are then looped from the surface of the dichromate to a microscopic slide by means of a small vial or the open end of a glass tube. They are then counted and classified according to the stages of development to which they have attained.

ARTHRITIS: Gland Cultures in Infectious, Poston, M. A. J. A. M. A. 93: 692, 1929.

By previous arrangement the necessary mediums and instruments are ready for the preparation of the cultures of the gland immediately after removal. If other work prevents immediate culture, the glands are moistened with sterile isotonic salt solution, previously warmed to 37° C., and placed in the incubator. The glands are rinsed twice with warm isotonic salt solution and ground in the sterile tissue press of special design.

Then 1 c.c. of beef-infusion-broth P_H 7.4 is added with a pipette while the ground gland remains in the press and suspension accomplished by blowing back and forth.

Two cubic centimeters of sterile human ascitic fluid and 0.2 c.c. of the gland suspension are added to each of two tubes containing 15 c.c. of molten beef-infusion-dextrose-agar P_H 7.4, cooled to 40° C. Each mixture is transferred to Noguchi tubes 14 by 200 mm. and overlaid with 1 c.c. of sterile petrolatum. The tubes are incubated at 37° C. and examined by transmitted light daily. All tubes are kept for six weeks before being reported as negative. To pick colonies from deep tubes the column of agar is transferred to a sterile Petri plate in the following manner: A sterile 8 mm. glass tubing with a 1 mm. thick wall is drawn out in the gas flame to make a capillary tube at least 25 cm. long; the large end is inserted into a 50 c.c. rubber pressure bulb and the capillary warmed by passing through a flame. The capillary is pushed between the column of mediums and the wall of the tube until the butt is reached. Pressure is applied to the bulb while the butt end of the tube is held over the flame. As soon as the column is dislodged, more air is forced into the butt and the column will slip easily by gravity into the Petri plate. The colonies are picked under a dissecting microscope and transferred to tubes of beef infusion blood broth, which are incubated anaerobically.

Parallel cultures from the gland are prepared by adding 0.2 c.c. of the suspension to rabbit blood agar plates, beef infusion dextrose blood broth and ascitic fluid dextrose agar slants.

Vaccines of these organisms consist of a forty-eight hour beef infusion broth culture heated to 60° C. for one hour. After heating, a culture is made from the vaccine to make certain that it no longer contains viable organisms.

LEUCOCYTES: The Polymorphonuclear Count in the Newborn, Sanford, H. N. Am. J. Dis. Child. 38: 547, 1929.

From a study of 100 infants the author concluded that short exposures to the ultraviolet light tend to increase the number of young or single-lobed nucleated cells of the polymorphonuclears. The older or multilobed forms are decreased. There is a rapid return to normal.

BLOOD COUNT: Relationship of Jaundice and Weight To Blood Values In The Newborn Infant, Mitchell, J. McK. Am. J. Dis. Child. 38: 518, 1929.

The erythrocyte count and hemoglobin content of the blood of 69 infants on the first, third, seventh, and tenth days of life are reported.

The percentage by volume of the cells in the blood of 50 newborn infants is also reported. The cell volume is considerably greater than the average value for adults. The volume index is also high.

Infants with jaundice do not show lower average erythrocyte and hemoglobin values than those without jaundice.

Practically all infants show a loss of erythrocytes and hemoglobin between birth and the tenth day of life. The average loss of infants with marked jaundice is only slightly greater than that of infants with no jaundice.

Relative changes in weight exert an effect on the loss of erythrocytes when the infants compared show the same degree of jaundice.

Infants with marked jaundice show a much smaller gain over their weight at birth on the tenth day of life than infants without jaundice. This exerts a leveling factor when one compares the loss of erythrocytes in the two groups.

Dehydration, when of a degree sufficient to be plainly manifest clinically and to cause fever, exerts a marked influence on the erythrocyte count.

Changes in percentage by volume in the plasma may be sufficient to prevent a closer correlation between the intensity of jaundice and the loss in erythrocytes and hemoglobin than shown in this series.

HEMOGLOBIN: Estimation of by Cell Concentration, Felsen, Jos. Arch. Path. 8: 484, 1929.

1. Shake a few grains of dry, powdered sodium citrate into a clean dry capillary glass tube having an inside diameter of 4 mm. and a length of 10 cm.

2. Holding the tube in the horizontal position or with the distal end slightly depressed, apply the proximal end to a bleeding wound. A rapid, deep, incised wound of the finger made with a sharp lancet-shaped needle will fill at least three such tubes with little or no pressure on the finger.

3. Fill the tube about two-thirds full and mix the blood with citrate by alternately elevating and depressing one end.

4. Allow the blood to gravitate to one end, seal that end with a plug of paraffin and place a broad rubber band snugly around the length of the tube, thus sealing both ends. It is well to file the cut edges to avoid injury to the fingers or tearing of the rubber band.

5. Centrifuge at high speed (plugged end down) for a sufficient length of time to secure the maximal separation of cells and plasma. This is determined as the period of time after which repeated centrifugation no longer diminishes the length of the cell column. Once established, the same button on the rheostat and the same period of time may be used with every specimen.

6. File-mark the capillary tube (a) at the junction of the paraffin plug with the cell column and (b) at the junction of the cell column with the plasma column. Break off at both points, thus isolating the cells.

7. By means of a Sahli pipette, graduated at 10 and 20 mm., the tip of which is applied directly to either of the open ends of the cell column, draw up the cells to the mark "10" (10 c.mm.). Add this to the graduated Sahli tube containing tenth normal hydrochloric acid to the mark 10. Wash the pipette thoroughly by sucking up and expelling some of the same fluid. Shake the mixture well, and then allow it to stand at least one minute or until the maximal change of color has been effected. Dilute with water to match the standard. When the Dare instrument is used, aspirate the cells from the capillary tube by means of a Sahli pipette, as before, to the mark "10." Then continue aspirating physiologic sodium chloride solution to the mark "20." Eject the resulting mixture (10 c.mm. of cells and 10 c.mm. of saline solution) on to a hanging drop slide or a small watch glass. Mix thoroughly by alternating aspirating and expelling the mixture from the pipette four or five times. Having secured a uniform mixture, take it up in the Sahli pipette and fill the Dare automatic pipette; the 20 c.mm. of fluid will completely fill the latter. Compare in the colorimeter.

The author concludes that when the cell-plasma ratio $\frac{c}{p}$ approximates 1 (i.e., when the cell column and the plasma column are approximately equal), the estimation of the percentage of hemoglobin by the cell concentration method yields a value most nearly approaching that obtained by the old method.

When the cell-plasma ratio is low (i.e., when the plasma content of the blood is relatively high), the new method gives a considerably higher percentage of hemoglobin than the old. This is least marked when the red cells are impoverished in hemoglobin while the red cell count is high.

In primary anemias, the cell-plasma ratio being low, the percentage of hemoglobin is found to be much higher than with the old method.

In chlorosis, the cell-plasma ratio being high, the percentage of hemoglobin is found to be the same or lower than with the old method, by reason of the low hemoglobin content of individual cells.

In polycythemia vera, the cell-plasma ratio being high, the percentage of hemoglobin is found to be lower than with the old method.

In secondary anemias associated with acute hemorrhage, the cell-plasma ratio being low, the percentage of hemoglobin is found to be either normal or low. This is due to the fact that cells are lost, rather than hemoglobin, the percentage of the latter being frequently normal as estimated by the concentration method. The difference between the old method

and the new is due to the fact that the latter eliminates one variable factor, the diluent plasma. The importance of this observation is evident in repeated examinations of a patient with bleeding gastric or duodenal ulcer. Watching the cell-plasma ratio will be found a much more reliable index than estimation of the percentage of hemoglobin and the number of erythrocytes.

In secondary anemias associated with dehydration (cachexia, inanition), the cell-plasma ratio being high, the percentage of hemoglobin will be found lower by the new method because the cells have already been concentrated in vivo.

These observations suggest a new concept of anemias. Anemias may be divided into:

1. Cytanemia: This classification embraces the majority of the types of anemias and is due to a diminution in the number of the red blood cells. It includes secondary and primary (pernicious) anemias. The number of erythrocytes is low, the percentage of hemoglobin is normal or almost so, the color index tends to be high, and the cell-plasma ratio is low (less than 1).

2. Hemoglobinemia: This classification includes some secondary anemias and chlorosis. Here the percentage of hemoglobin is low, the number of erythrocytes is normal or increased, the color index is 1 or less and the cell-plasma ratio is 1.

It is interesting to note that in polycythemia vera the percentage of hemoglobin is normal, the number of erythrocytes is greatly increased, the color index is 1 or less and the cell-plasma ratio is high (more than 1).

Some secondary anemias and aplastic anemia fall in an intermediate group between 1 and 2. The foregoing simple classification of anemias is based on the determination of whether the cells or the hemoglobin are primarily involved. The use of the cell-plasma ratio and the estimation of the hemoglobin content by the cell concentration method appear to be satisfactorily adapted for such a procedure.

ANEROBES: Simple Method for Isolation of, Wenzel, T. Arch. Path. 8: 487, 1929.

For the culture of blood, 2 c.c. of the citrated specimen is added to a tube containing about 15 c.c. of melted agar, and the mixture poured into a Petri dish. After inoculation, the plate is chilled in the ice box until the agar is firm; sterile melted petrolatum is then poured over the surface of the plate to give a layer about 1 cm. in depth. For the culture of surgical swabs, pus and other material, the same procedure is used with the exception that plates are prepared with several different dilutions of the test material to insure obtaining discrete colonies.

The plates are incubated in the usual way.

No difficulty is experienced in the detection of colonies on positive cultures. When it is desired to pick the colonies for transplanting or for staining, the plate is chilled and the hardened layer of petrolatum is easily lifted away with a sterile wooden tongue depressor. If the plate contains gas-formers, it is best to make transplants before the production of gas causes disruption of the culture medium and bubbling of the petrolatum layer.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Vaccination Against Tuberculosis by Means of B. C. G.**

IN VIEW of the widespread interest and discussion concerning the work of Calmette and Guerin in the prophylaxis of tuberculosis by vaccination, this pamphlet will be read with interest by all who are at all concerned with this problem.

It represents the findings of three commissions, Bacteriologic, Clinical, and Veterinary, and presents concisely a rather comprehensive survey of this question.

The Bacteriologic Committee, with one exception are agreed that B. C. G. is a harmless vaccine and does not give rise to progressive tuberculosis.

Prof. Nobel, however, believes that under exceptional conditions B. C. G. was capable of inducing fatal tuberculosis in laboratory animals.

The following studies were deemed desirable:

1. Methods to be adopted in order to maintain unaltered the fixed properties recognized as characteristic of B. C. G.

2. Methods to be adopted for the study of the influence on B. C. G. of passages through animals.

3. Methods to be adopted for immunizing experiments; determination of doses of B. C. G. and virulent bacilli to be used in them; adoption of strains of known virulence for the virulent inoculations.

4. Methods to be adopted in the studies on the variability and dissociability of B. C. G.

5. Methods to be adopted for the comparative study of the histologic changes produced by inoculation of B. C. G. and of virulent tuberculosis bacilli.

6. Necessity of entrusting the preparation of B. C. G. (culture make and distribution of vaccinal emulsions) to institutes of recognized scientific standing.

The Clinical Commission from the documentary evidence laid before it concluded that:

1. B. C. G., when administered per os to infants within ten days after birth, or hypodermically to older children and adults, was incapable of producing virulent tuberculosis lesions.

2. As regards the preimmunizing properties of B. C. G. against tuberculosis, a certain degree of immunity was induced by this vaccination.

Further research work upon vaccinated children, continuing for a more lengthy period and carried out in a uniform manner—and, in particular, a fuller knowledge of tuberculosis morbidity and mortality among individuals of varying age and environment—are necessary before the Commission is able to pass a final judgment on the value of antituberculosis vaccination with B. C. G.

The conclusions of the Veterinary Commission were that:

1. The mass of experimental data published and the unanimous opinion of practitioners who have used B. C. G. upon bovidae indicate that vaccination performed according to the technic advocated by Calmette and Guerin upon animals of this species is entirely innocuous.

*Report of The Technical Conference for The Study of Vaccination Against Tuberculosis By Means of B. C. G., League of Nations Health Organization, 1928; Paper, 147 pages. World Peace Foundation, Boston.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

2. The same experimental data and the observations made of the practical use of B. C. G. upon bovidae show conclusively that this strain of bacilli possesses preimmunizing properties against both experimental and natural tuberculous infections.

These acknowledged preimmunizing properties provide justification and encouragement for the extension of the experimental use of B. C. G. in the prophylaxis of bovine tuberculosis.

The use of B. C. G. should be continued in the various countries in accordance with the international experimental protocol which is attached, under the supervision of official veterinary services and competent bacteriologic and pathologic authorities, and in close touch with the Commission set up for the study of this problem by the Health Organization of the League of Nations.

Wherever possible, the trials should be carried out strictly in accordance with the experimental rules; in other cases, trials may be made more in conformity with the ordinary conditions of cattle rearing, under continuous official supervision.

The methods and plans for the further studies deemed necessary are set forth at length in the body of the report.

There is but one criticism: These reports, while of necessity temporary and to be supplemented by further data before final conclusions can be reached, are nevertheless of great value as sources of reference and as such should be available in somewhat more durable form.

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*Outline of Preventive Medicine**

"PREVENTION of disease," says Dana in the Foreword of this book, "involves at least two lines of action, one preventing the contraction of certain diseases, the other preventing the development of serious symptoms of the disease contracted. . . ."

Thus "prevention of disease often means the prevention of the disturbing symptomatic effects of a fundamental specific disease invasion."

And, again: "The usual attitude of the general practitioner toward preventive medicine is of course commendatory but not exactly enthusiastic."

The reviewer would suggest that to no small extent this comparative indifference may be due to some small lack of clear understanding of the situation and its ramifications. It might be taken as a somewhat striking commentary upon medical practice in general that, following the propaganda concerning the periodic health examination, it should have been felt necessary to demonstrate by moving pictures, etc., how to make a thorough physical examination.

This fact, it seems, is deserving of some thought. Either such demonstrations were insulting to the rank and file or, the rank and file are due to consider whether they are competently practicing what they preach.

This book, it seems, to the reviewer, fills a needed want. Simply written, yet authoritative, its perusal should arouse thought and give impetus to the necessary study required to practice preventive medicine in the fullest sense. It may be read with profit by physician and layman alike.

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*Outline of Preventive Medicine, by 21 contributors, edited by T. E. Sondern, C. G. Heyd, and E. H. L. Corwin. Cloth, 398 pages, Paul B. Hoeber Inc., New York.

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EDITORIALS

VICTOR C. VAUGHAN (1851-1929)

AN APPRECIATION

ON NOVEMBER 21, 1929, twenty-five days after his seventy-eighth birthday, death came to Victor C. Vaughan as it must come to the great as well as to the small. A full life of absorbing interest, active, useful, long, and well-rounded, which gives proof of his accomplishments, has been closed. Silenced is the convincing voice and halted the powerful pen that has wrought so effectively in the renaissance of science of the past half century. Exceedingly well done is the universal characterization of his work in every phase of the broad field that he covered, whether as teacher, chemist, toxicologist, investigator, sanitarian, medicolegal expert, organizer, administrator, medical educator, author, or editor, historian, and finally philosopher.

High positions and honors, the acclaim of the scientific world, of governments, of the army, had been heaped upon him, but most precious of all to

him was the esteem of other great men and the love of his students. His family life was exemplary and he inculcated in his sons high ideals. It was his happy lot to live to see his work appreciated and his sons carry on successfully.

The World War brought three colonelcies, a majority, a captaincy, and a lieutenancy to his family, but it also caused the one shadow of his life when it took from him his first-born son. The sorrow of this irreparable loss shattered the indomitable spirit of the man; he aged in spite of his will to carry on. He gradually withdrew from the more intense activities of life.

In 1921 at the age of seventy he resigned from his duties in the Medical School in the University of Michigan. It was a sad parting from his Alma Mater, the great institution that he had builded. He still had much to do in his chairmanship of the National Research Council, in launching *Hygiea*, in putting this, his own JOURNAL OF LABORATORY AND CLINICAL MEDICINE into order, and in writing the important story of his life, *A Doctor's Memories*. In his usual, interesting, charming way he penned the scenes that had passed by him in his long, active journey through life. The panorama of American medicine and the outstanding men of his generation have been accurately and faithfully described by him as he saw them with his own quick analytical senses in close personal contacts.

Doctor Vaughan aroused in his students sentiments that are too hallowed to entrust to inadequate words. It would be a Herculean task to recount all of the deeds and accomplishments, the works and honors of a life that has been so extraordinarily full. A decision as to which of his attainments may be commented upon as his greatest depends upon what contact his biographer has had with him. He is outstanding in many fields. To his students, however, and to the great mass of medical men he was primarily a teacher and a preeminent pioneer in medical education. He has brought his stimulating influence to bear upon his colleagues as well as upon each individual of the mass of undergraduates who passed through his classroom or under his tutelage through the thirty odd years that he served successfully as professor of physiological chemistry and hygiene and dean of the Medical School of the University of Michigan. This institution is a monument to his genius, for here, in a small town, he built a great medical school and maintained it in the first rank throughout his years as its dean. It still shines resplendent in glory of its past. He possessed an uncanny ability to choose his faculty members wisely and to organize and administer the school. Frequently men whom he had developed were taken from him by the heavily endowed institutions in the East, to one of which he had to relinquish no less than seven of his men, but he was always able to rebuild satisfactorily. A splendid nucleus of home talent he was finally able to hold against all inducements.

In the eyes of his students from the first day of classwork, Doctor Vaughan was the quintessence of medical scientists. He somehow inspired a deep sense of reverence and had a tendency to inspire hero worship, although he himself was never given to such. His was a very critical attitude of mind, but a sense of humor was its saving grace. He was sharply critical of himself as well as of others. He usually held himself in reserve, and successfully

mastered his inner thoughts. He insisted upon the three I's, industry, intelligence, and integrity. He lived up to the demands that he made of others. Deep sincerity of purpose and action were other marks of his greatness.

He traveled the highways of life with the great men of his age and has been able to count many of them among his intimate friends. Respect of the great stimulated him, but he lost not the common touch. His contacts with his students meant most to him. He always divided his classes into the sheep and the goats, but a sincere fatherly interest was with the individual even though he were among the goats. His outward show was not so fatherly as that of others, but in the storm and stress periods the students soon learned of the sincerity of his friendship. His was a personal contact with his "boys," each of whom he called by his Christian name. He always remembered some anecdote concerning each one, which he would relate at an opportune moment.

Friendship was to him sacred. He never grew blasé with the honors that were heaped upon him. He attributed whatever success he had attained to the loyalty of his friends. Inwardly he was a most modest soul. His outward bearing was never that of a feigned modesty; in fact, it was always such as to mask his feelings completely. Only the few, to whom years of association had given glimpses beyond the front, were able to realize his sensitive self-critical attitude. He was an aristocrat in the true sense of the word.

A great privilege and honor it has been indeed to know such a spirit and to have come under such an influence as his, to have been advised and guided by his genius. He has made a lasting impression upon the medical men of his generation. His job is done and well done.

The founding of this JOURNAL OF LABORATORY AND CLINICAL MEDICINE was simply an outgrowth of his pioneer work in which his vision and foresight perceived the importance of the laboratory in clinical medicine. The greatness of this vision has been justified. Such is the expression of our sentiments, however futile and inadequate our words may be.

—George Herrmann.

VICTOR C. VAUGHAN

An Appreciation

THE artist can neither paint the sunset nor gild the rose quite so well as he would like. The builder never creates with steel and stone quite so perfectly as he dreams. The writer never makes the children of his fancy so real as he desires. The worshiper never gives to his hero the halo that he believes the hero deserves. For these reasons, I am quite unable to pay the homage to Doctor Vaughan that my heart yearns to pay.

Doctor Vaughan was born in Mount Airy, Missouri, seventy-eight years ago, and spent his boyhood not far from where another great Missourian, Samuel W. Clemens, Mark Twain, passed his youth. The lives of both these men have proved to be a blessing and a benediction to mankind: the one for the undying service rendered his fellow men in the fight against disease; the other for the joy brought to countless millions through the written word. No crusader ever fought with a greater zeal than did Victor Vaughan in the practice and in the teaching of medicine. No one better understood the problems of the general practitioner than he. No one worked harder than he to help the general practitioner to solve them. Unlike many of his great contemporaries—Osler, Lister, Welch, Pasteur, Koch, Halsted—Doctor Vaughan's labors were not confined so much to hospitals as to the outside cases. In the homes to which he was called in counsel by the family physicians he did his most effective work. These contacts burned into his soul the knowledge that better trained general practitioners were needed, and this need was the urge that drove him continuously on and on to build better and equip more scientifically until he made the Medical Department of the University of Michigan, of which he was dean for thirty years, one of the great medical schools of America.

Like most empire builders, Doctor Vaughan was blessed with a vivid imagination. He saw far into the future; then, with a stout heart and willing hands he labored long and earnestly to realize his dreams. Never shall I forget an evening spent with him in the library of his home at Ann Arbor, listening to the story of when and how he first conceived the idea that the common house fly was a carrier of the deadly typhoid fever germ. "During the Spanish American War," said he, "I was sitting in the officers' quarters of the training camp in Chickamauga. Typhoid fever was raging. I had just come from the hospital where hundreds of patients with typhoid fever were under treatment. I had seen the latrines into which had been dumped the sewage from the hospitals. These latrines literally swarmed with flies. Why could not these flies be the same ones that I saw walking on the edges of drinking cups, cooking utensils, and even on food in the officers' quarters? Turning to a fellow medical officer I said, 'Doctor, these flies may be carrying on their feet enough typhoid germs to kill us all.'" Subsequent investigation has proved the correctness of this theory.

One who was privileged to sit at his feet can well understand how he could draw medical students to him and inspire them. His earnestness, his integrity, his zeal for service were contagious. His smile quickened the pulse, buoyed the spirit, renewed the courage, and brought greater hope to all who came in contact with him. It is not surprising that the graduates of the University of Michigan have been pathfinders in medicine in many lands, in many climes. They are carrying on as the old master bade them, and they will continue to carry on in his name while there is one alive to revere his memory.

Preventive medicine was the most alluring field to him. "To keep people from getting sick is the goal of the true physician," said he. It was his great ambition to see a county hospital in every county in every state, and in these hospitals a well-trained, competent staff willing and ready to serve. One of the most inspiring articles he ever wrote, "The Doctor's Dream," made vivid mention of these things. Doctor Vaughan abhorred medical politics. The Star Chamber sessions at state or national medical meetings for the purpose of furthering some ambitious doctor's dream for power had his profound contempt. Almost every gift that organized medicine could bestow on one was given him, but they came unsought.

To him more than to any other American physician credit is due for the place the medical laboratory now occupies in scientific medicine. In the October, 1915, issue of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE he said editorially, "The man who attempts to practice medicine without laboratory aid, belongs to a past generation and fails to do justice to his patients or credit to himself."

He lived to see well-equipped, medical laboratories a part of every correctly managed hospital, group clinic, and private office. He lived to see more accurate diagnoses made and better medical service rendered to millions of people because of his teaching in this field of medicine. Much is being said and written today in scientific medical circles about allergy. An understanding of this phenomenon has taken much of the mystery away from certain diseases. Years ago Doctor Vaughan with prophetic vision predicted this. His work on protein poisons was the forerunner of present-day understanding of allergic reactions. Here, again, he lived to see his dreams come true. He, more than any other authority, counseled against the indiscriminate use of vaccines. When manufacturers were making stock vaccines and physicians were using them with but little understanding of their danger, his voice was raised against this practice. Time proved the logic of his deductions; but his was the voice in the wilderness crying out against such methods. Once Doctor Vaughan reached a conclusion, he was not afraid to defend it. To this attribute much of his greatness can be ascribed.

It can well be said of him that he walked with kings but never lost the common touch. An appeal for help from the most obscure country doctor for a patient unable to pay was given the same sympathetic consideration as one that came from the crowned heads among the medical aristocracy of America or Europe.

The unknowable, the mysterious, the baffling in medicine made a strong appeal to him. It was a challenge that he accepted and clung to until the light

dawned. This quality made him the great epidemiologist that he was, probably the greatest of his day. In peace or war, when an epidemic raised the black flag of death and sought to strike men down, his was the mailed fist that came to the rescue.

No more kindly, congenial spirit ever blessed the dwelling place of man than Doctor Vaughan. He was intensely human, a good companion, wise counselor, loyal friend, and willing at all times to accept the fortunes of combat. Neither victory nor defeat swerved him from the even tenor of his way.

Easily the outstanding toxicologist and medical jurisprudence expert of his time, his great talents were never for sale to the strong to be used against the weak. He resented the technicalities so often resorted to in the courts for the purpose of defeating justice. This resentment caused him to retire voluntarily from this field after a noted murder trial in Kansas City in which he felt that justice had sadly miscarried. Great as Doctor Vaughan became in the field of science, this made not the slightest difference in his relationship with his fellow men. Nothing but gentleness, kindness, brotherly love, and quiet marked his hospitality which was so much in evidence in his Ann Arbor home. Here he met the great and the small in medicine, and all were treated alike. The renowned professor, the humble medical student, the struggling country doctor, or even the wayfarer in the commercial desert whose wanderings brought him to Ann Arbor for a conference with Doctor Vaughan—all found a cordial welcome, and all left inspired and impressed.

Death as well as birth is a natural course. Short indeed is the race from the cradle to the grave. The eye grows dim, the heart is saddened over the passing of so great a man; but with this grief there comes a gladness in the realization that one such as he has lived to be a husband, a father, a teacher, and a friend.

—*C. V. Mosby.*

The American Society of Clinical Pathologists

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News and Notes

Dr. J. J. Moore, Chicago, Illinois, presented a paper entitled "The Relation of the Record Librarian to the Clinical Laboratory" before the Convention of the Association of Record Librarians of North America, October 14 to 18, 1929, at Chicago, Illinois.

The Official Representatives of the American Society of Clinical Pathologists to the Nineteenth Clinical Congress of the American College of Surgeons at Chicago, October 14 to 18, 1929 were as follows: Dr. Oliver W. Lohr, Saginaw, Michigan, Dr. Frank W. Hartman, Detroit, Michigan, Dr. William Thalhimer, Chicago, Dr. H. C. Sweany, Chicago, Dr. J. J. Moore, Chicago.

Dr. Frank W. Hartman, Detroit, Michigan, presented "What Constitutes an Efficient Clinical Laboratory Service for a Hospital?" on the program of the Twelfth Annual Hospital Standardization Conference held in Chicago, October 14, 15, 16, and 17, 1929. Dr. Oliver W. Lohr, Saginaw, Michigan, opened the discussion of this paper.

Dr. Charles R. Drake, Minneapolis, Minnesota, and Dr. Kano Ikeda, St. Paul, represented the Society at the Fifty-eighth Annual Convention of the American Public Health Association in Minneapolis, September 30 to October 5, 1929.

The Board of Registry of Technicians of the American Society of Clinical Pathologists has begun issuing certificates to those technicians successfully meeting the requirements. These diplomas are being sent out as quickly as the investigation of the applicant is completed. The Placement Bureau has been operating very successfully and several technicians have been placed in desirable positions. You are invited to take advantage of the facilities offered by this department of the Registry